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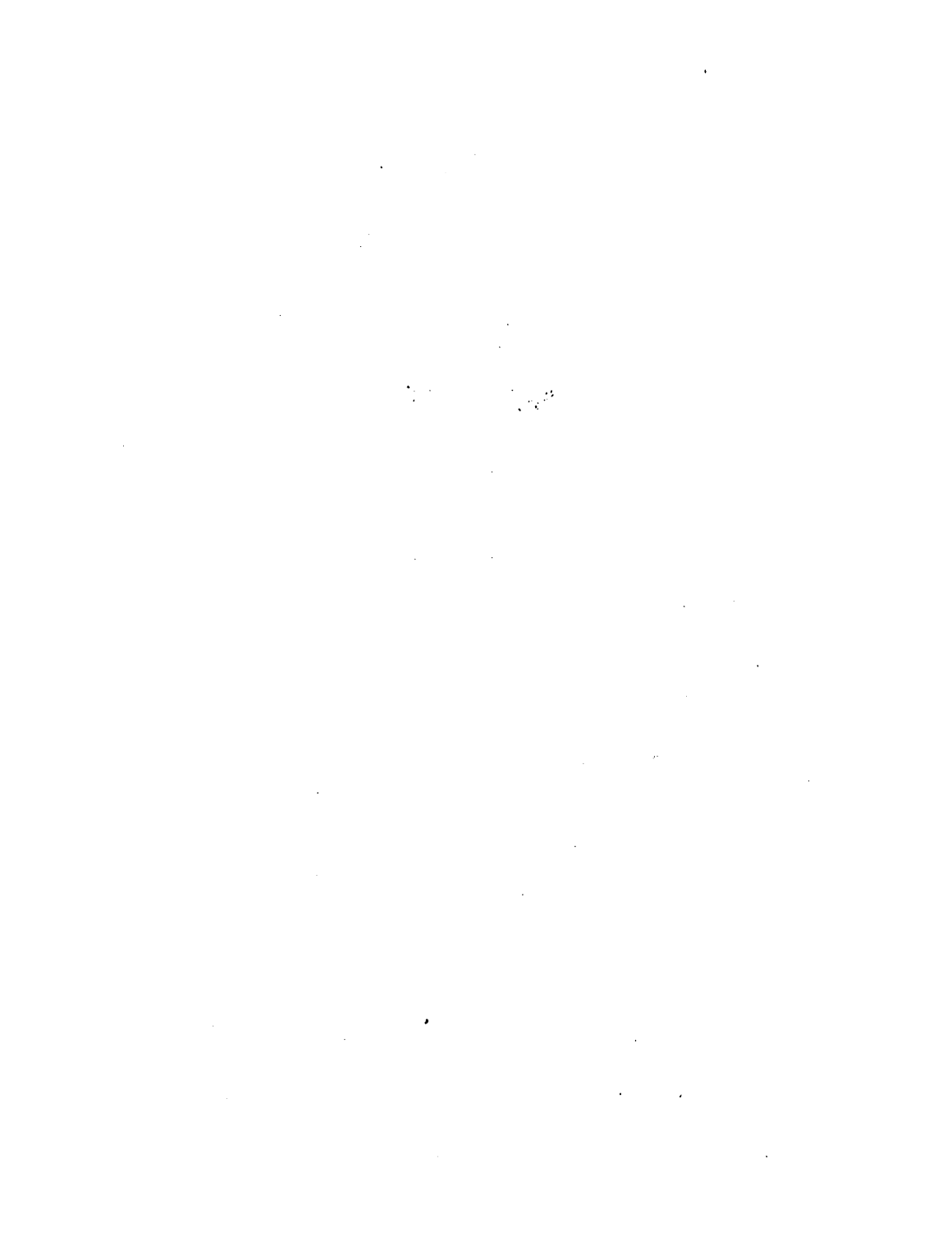
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**BIOLOGICAL
LABORATORY METHODS**

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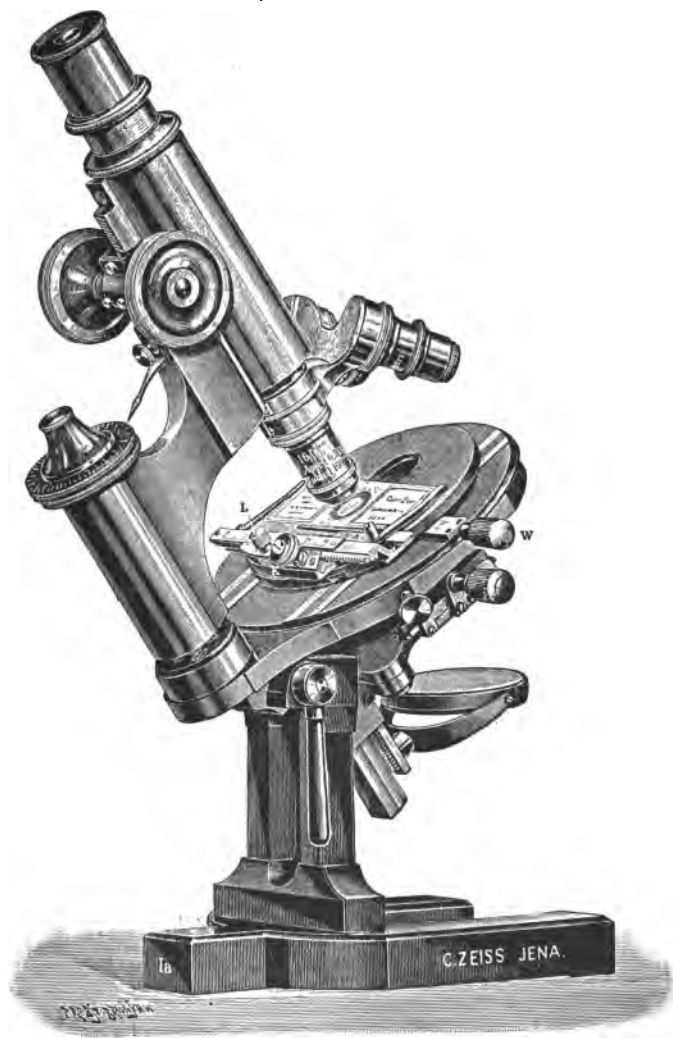


PLATE I.

BIOLOGICAL

LABORATORY METHODS

BY

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INSTITUTE



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CONTENTS

	PAGE
INTRODUCTION	xi
CHAPTER I	
THE MICROSCOPE	I
CHAPTER II	
OCULARS AND OBJECTIVES	21
CHAPTER III	
APPARATUS AND ACCESSORIES	45
CHAPTER IV	
PREPARATION OF THE TISSUE FOR MOUNTING	66
CHAPTER V	
IMBEDDING METHODS	83
CHAPTER VI	
STAINS, THEIR PREPARATION AND USE	105
CHAPTER VII	
MOUNTING THE TISSUE FOR PRESERVATION	127
CHAPTER VIII	
HOW TO MAKE DRAWINGS OF THE SECTIONS OF TISSUE	132
CHAPTER IX	
PHOTO-MICROGRAPHS. THE APPARATUS	141

CHAPTER X	
MAKING THE PHOTO-MICROGRAPHS (CONTINUED)	PAGE 156
CHAPTER XI	
PHOTO-MICROGRAPHS (CONTINUED) — SUNLIGHT AND ELECTRIC LIGHT, OXY-HYDROGEN, ACETYLENE, CITY GAS, AND OIL LAMPS, AND HOW TO USE THEM	165
CHAPTER XII	
PHOTO-MICROGRAPHS (CONTINUED) — PHOTOGRAPHIC DRY PLATES AND MAKING THE NEGATIVES	173
CHAPTER XIII	
MAKING THE POSITIVES	186
CHAPTER XIV	
APPARATUS AND METHODS IN THE STUDY OF BACTERIOLOGY	200
CHAPTER XV	
BLEACHING, DECALCIFICATION, INJECTION, MACERATION	225
CHAPTER XVI	
POLARIZATION OF LIGHT AND ITS APPLICATION TO BIOLOGICAL IN- VESTIGATIONS	252
CHAPTER XVII	
USEFUL FORMULÆ AND TABLES	260
APPENDIX	
THE ARRANGEMENT OF THE LABORATORY AND ITS FURNITURE	305
INDEX	315

ILLUSTRATIONS

	PAGE
Apparatus for removing Air Bubbles from Slides	295
Anaërobic Apparatus, Novy's	222
Anaërobic Culture, Kaspavec's	221
Anaërobic Culture, Wright's	281
Autoclave	207
Bleaching, Marsh's Chlorin Method	227
Box for Lantern Slides	195
Camera, Kodak Cartridge	142
Camera, Large Photo-micrographic, Zeiss's	163
Camera, Small Photo-micrographic, Zeiss's	160-161
Camera Lucida, Bausch & Lomb	135
Camera Lucida, Section, Abbe's	134
Camera Lucida, Abbe's	133
Chromatic Aberration	5
Coddington Lens	2
Counting Apparatus	223
Cover-glass Circles	128
Cover-glass Squares	128
Cover-glass Gauge, B. & L.	29
Cover-glass Gauge, Zeiss's	30
Dehydrating Apparatus, Thomas's	59
Developing Tray	151
Diaphragm Shutter, Iris	148
Dissecting Microscope, Zeiss's	3
Dissecting Needles	55
Dissecting Scalpel	56
Dissolving Key for Lantern	198
Drawing-table, Bernhard's	136
Drawing-table, B. & L. Modification of Bernhard's	137
Electric Lamp for Lantern, Colt's	168
Eyepiece Micrometer	27
Film Cartridge	145
Fixing-bath, Gennert's Universal	151

	PAGE
Flasks, Erlenmeyer's	59
Flasks, Koch's	59
Forceps	54
Freezing Attachment	98
Glass Benches	57
Graduate	151
Hanging-drop Culture	219
Illuminating Apparatus, Abbe's	16
Incubator, Laboratory	208
Injection Apparatus, Sterling's Constant Pressure	235
Laboratory Tables	208-209
Lamp, Carbutt's Multum in Parvo	149
Lamp, Acetylene	172
Lantern, Colt's Criterion with Electric Lamp and Microscope Attachment	197
Lens of Crown Glass and Flint Glass	6
Mat for Lantern Slide	194
Microscope, Section of	9
Microscope, Continental BB	7
Microscope, Stand Ia with Mechanical Stage, Zeiss's	<i>Frontispiece</i>
Microscope, Leitz's	11
Microscope, Spencer's	13
Microtome, Hand	46
Microtome, Automatic	47
Microtome, Minot's Rotary	48
Microtome, Minot's Precision	50
Microtome, Ribbon Carrier	49
Microtome, Reichert's	51
Microtome Knives	52
Microtome Knife-holder	52
Microtome, Freezing, Section of	95
Microtome, Jung's Freezing	97
Needle-holders and Needles	55
Negative Washing-bath	152
Negative Drying-rack	153
Nivellating Apparatus	220
Nose-piece	19
Objectives	31
Objective, Section showing Oil Immersion	32
Objective, Section of Apochromatic	37
Oculars, Compensating	22
Oculars, Continental	21

	PAGE
Oculars, Huyghenian	21
Oculars, Micrometer	27
Oculars, Filar Micrometer	27
Oculars, Projection	26
Paraffin Imbedding Box	64
Petri Dish	58
Pipette	58
Polarized Light	253
Polarizers, Zeiss's	254
Pressure Gauge	171
Printing-frame	153
Ray Filter	150
Retouching-frame	153
Roll-holders, Cartridge	144
Rheostat, Colt's	169
Scissors, Dissecting	54
Section Lifters	57
Sliding Objective Changers, Zeiss's	19
Slides, American	128
Slides, German	128
Spherical Aberration	4
Staining-jar, Naples	109
Staining-dish, Moore's	109
Sterilizer, Hot-air, Lautenschlaeger's	204
Sterilizer, Hot-air, B. & L.	203
Sterilizer, Steam, Arnold's	205
Sterilizer, Steam, Section of Arnold's	206
Swing-out Condenser, Zeiss's	17
Swing-out Condenser attached to Microscope, Zeiss's	18
Syringe, Stroschein's Injection	235
Syringe with Cannulas	234
Thermo-regulator	210
Tripod Case	143
Tripod, Folding	143
Turn-table	60
Water-bath, Lillie's	63
Water-bath, Reeves's	62
Water-bath, Miller's	61
Watch-glasses	58
Wire Baskets for Culture Tubes	209



INTRODUCTION

THE establishment of the Experiment Stations in the several states of the Union in connection with the colleges has given such a strong impetus to scientific research that no institution is considered to be well equipped unless it has a laboratory for biological investigation, and a course of work in this laboratory made a part of the curriculum of the college. The demand is therefore becoming greater each year for suitable text-books which will give full and clear instructions concerning the use of the microscope and the other instruments and methods required in these laboratories. It is true there are excellent works in existence which treat of the microscope and the methods in vogue among investigators, but these books are generally in such shape as to render them suitable simply for works of reference. For they are either too voluminous or devoted to only one or a few of the many subjects, it is required of a student in biology to know, and they are thus rendered unsuitable for text-books; and they take up the subject in many instances where the beginner has insufficient knowledge of the topic to follow the author. A text-book for students, particularly in practical work, should begin at the beginning and treat of all matter relating to the subject in simple language, and in sufficient detail to convey to the mind of the student a clear comprehension of the work to be required at his hands.

The editor of the *Journal of Applied Microscopy* in a recent issue of this most excellent paper has clearly stated the matter when he says: "Books which begin at the beginning, and which deal with facts in a perfectly scientific, accurate manner, and yet with the utmost simplicity, which will aid the beginner without confusing, which will develop his powers of observation

without detracting from the thing itself, and which will tell him the what, where, and how of microscopical work, will be appreciated by the thinking laity."

In the preparation of this book the author has also been guided by what seemed to be the average advancement of the students who report from year to year for admission to the scientific courses in the Alabama Polytechnic Institute. The topics treated in the following pages have not been greatly elaborated, but the discussions have been conducted far enough to give something more than simply suggestions and outlines. It is expected that the student will build only the foundation in the study of this book, but it is hoped by the author that the information contained herein will be the incentive for inducing the student to extend his investigations in the domain of biological science until he becomes proficient in research and is able to contribute to the knowledge of the subject.

In writing this book liberal use has been made of the standard works relating to microscopy and to histology, and much valuable information has been secured from the papers and periodicals devoted to the science of biology.

The following firms have placed the author under obligations for permission to use electroplates from which some of the illustrations have been produced: Bausch & Lomb Optical Company; Carl Zeiss Company; Eastman Kodak Company; Buffalo Dental Manufacturing Company; and Rochester Optical Company.

P. H. MELL.

AUBURN, ALABAMA,
October, 1902.

**BIOLOGICAL
LABORATORY METHODS**



BIOLOGICAL LABORATORY METHODS

CHAPTER I

THE MICROSCOPE

THE microscope, an essential instrument in every laboratory, should be well made, out of the best material, to enable the student to perform satisfactory work and secure accurate results.

There is no economy in purchasing a "cheap" microscope, but great care and experienced judgment should be exercised in providing each student with a first-class instrument made by the most reliable manufacturer. This important fact being well understood and appreciated, it then becomes a matter largely controlled by taste and the character of the work which instrument, from the list of less than a dozen responsible opticians, it is advisable to purchase. In this country and abroad excellent microscopes are made by such houses as Bausch & Lomb Optical Company, Zeiss, Leitz, and others.

The cost of the microscope will increase in proportion to the delicacy of the work required, the minuteness of the object under investigation, and the advancement of the student in histology. So that, after determining upon the pattern of stand, the most important question to be answered is what kind and number of objectives and oculars are required. As a general rule it is best to begin with two or three objectives with oculars to match, and add to the list as the needs demand.

Each student should be provided with

1. A Simple or Dissecting Microscope.
2. A Compound Microscope.

The dissecting microscope is a convex glass lens mounted in a metal or rubber holder and attached to a convenient stand with rack and pinion adjustment, mirror and stage, so that the object may be placed under the lens, a steady focus secured, and the hands left free to manipulate the dissecting instruments with the least degree of inconvenience. The image produced by this instrument is not inverted as it is in the compound microscope, and hence the dissection can be performed with much greater ease.

For a long time the single glass employed in the earlier history of this instrument produced spherical aberration and aberration of refrangibility to such a great degree it was found necessary to replace the single lens with what Wollaston, the inventor, termed *doublets*. The two lenses, or doublets, in Wollaston's instrument are so adjusted in relation to each other that they act together as a single glass. They are plano-convex and are separated by means of a diaphragm. Besides overcoming, to a large extent, spherical aberration, by the use of two glasses with a diaphragm interposed, the doublets also increase the distance between the lens and the object, thus permitting more freedom in the manipulation of the dissecting instruments.

Achromatic triplets may also be used on the microscope, thus giving a large, flat field and a beautiful definition.

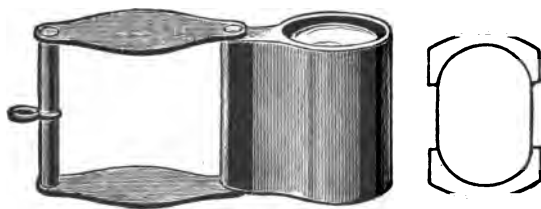


FIG. 1. — Coddington Magnifier.

Coddington magnifiers (Fig. 1) are convenient for field work and are highly prized by all practical botanists. The figure represents an aplanatic triplet lens which is achromatic. It is composed of two flint lenses between which is cemented a thick crown

glass. The magnification is about ten diameters, and the image is free from distortion.

A most excellent dissecting microscope is made by the Zeiss Optical Company of Germany and is illustrated in Fig. 2. The manufacturers in describing this instrument say:—

“The stage consists of a large metal frame (10×10 cm. = 4×4 in.), to which is attached wooden folding hand-rests; adjustment by rack and pinion, plane and concave mirrors with

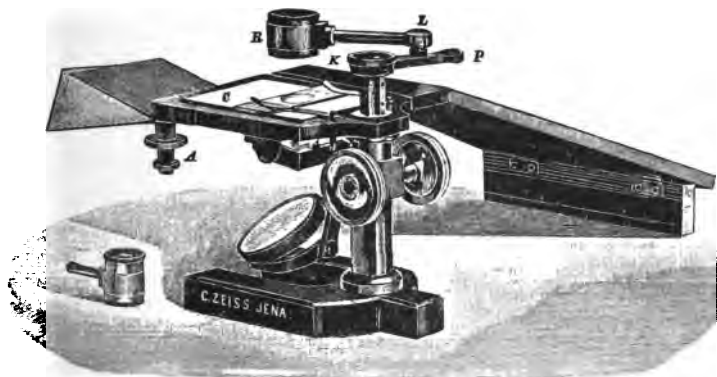


FIG. 2. — Zeiss Dissecting Microscope.

universal motions, to which a piece of white paper may be attached by means of a ring so as to produce with low magnifications diffuse illumination.

“The triple dissecting system” which consists “of three achromatic lenses (objectives) and an achromatic concave eyepiece, forms a suitable combination for dissecting and ‘teasing’ small objects on a slip or in a watch-glass; it may be fixed in the ordinary lens-holder *P*, and a black metal plate with stage opening of 14 mm. diameter, which can be closed below by a black or white disk, may be placed under the object into a recess provided in the stage-frame.

“For examining large objects, particularly living aquatic animals, the aplanatic lenses will be found useful. These glasses

are composed of three cemented lenses, giving relatively long focal distances with large flat field. They fit into a separate arm LR , which may be inserted into the ordinary lens-support at L , and, by this arrangement, the whole of the stage can be scanned.

"In this case the metal stage is replaced by a glass plate, and the interchangeable white and black disks serve as convenient means of forming a white and black ground.

"A brass plate made to fit the frame serves as a support for small dissecting dishes, which may be cemented to it with paraffin."

Spherical and chromatic aberrations.—A few words in reference to this subject may not be out of place in a book of this

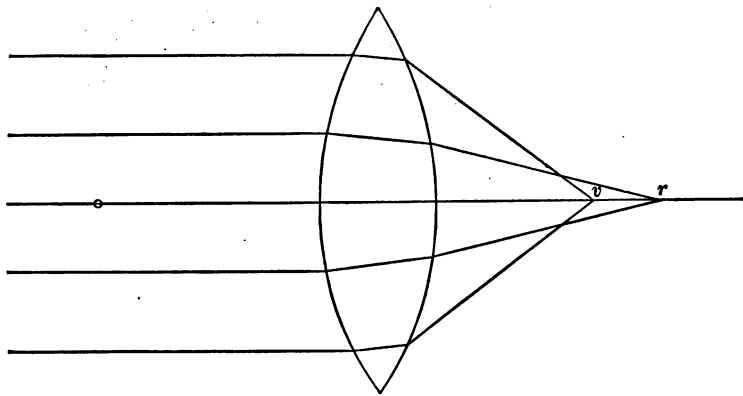


FIG. 3.—Spherical Aberration.

character in order that the student may form some general idea concerning the principles upon which the Wollaston lens is constructed. But, for a full and detailed account of refraction of light and correction of spherical and chromatic aberrations, he is referred to works on optics.

Spherical aberration, so common in single lenses, is due to that property a glass medium has of refracting the rays of light to a greater degree near the circumference than toward the centre of

the glass. For this reason many foci are formed, and the image becomes confused and indistinct. The rays near the axis of the lens and parallel to it are refracted when they penetrate the lens and are brought to a point in the principal focus; while those rays which enter the lens near its edges are refracted more and are focussed at a point nearer the lens. The formation of these foci by the same pencil of light is termed *spherical aberration*. Optical glasses producing this defect are not suitable for micro-

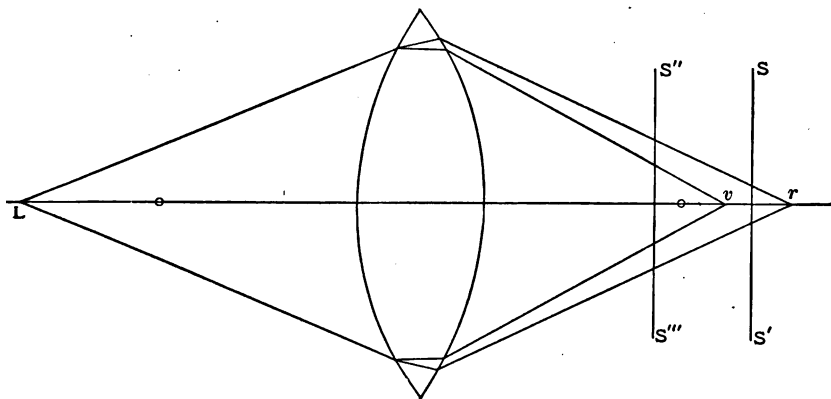


FIG. 4. — Chromatic Aberration.

scopic work, and the trouble must be overcome before objectives and oculars are sent out by the manufacturers. There are two ways of correcting such aberration: (1) by using a diaphragm which partly corrects the aberration by interrupting the scattered rays coming through the edges of the lens and permitting only those rays penetrating the lens near its axis to produce the image; (2) by so grinding the lens that its curvature near the edges is less than that near the centre. When a lens is corrected for spherical aberration it is called *aplanatic*.

Chromatic aberration refers to the decomposition of the rays of light into colors when they pass through a lens. This is also a serious defect which must be corrected as far as possible if the

objective is expected to do first-class work. It is a well-known fact to physical students that, of all the colors produced by a ray of light which is refracted through a prism, the violet color (its index of refraction being 1.5466) suffers the greatest refraction, while the red (its index being 1.5258) at the other limit of the spectrum submits to the least refraction. These two rays, therefore, after separating through the medium of the glass, meet the principal axis of the lens at different points, the violet nearest the glass, at v , and the red at r , farthest removed from the lens. A screen placed at ss will have projected on it a red ring at those points where the red ray strikes; and if the screen is removed to a position between v and the lens, two colored rings will be formed, the outer one will be red and the inner one violet. When an object is viewed through a lens giving such results, all colors of the rainbow are discernible, and the image becomes very much confused and indistinct, as it is with lenses producing spherical aberration. To correct chromatic aberration two different kinds of glasses must be used in making the

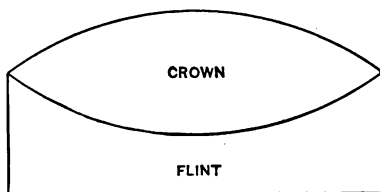
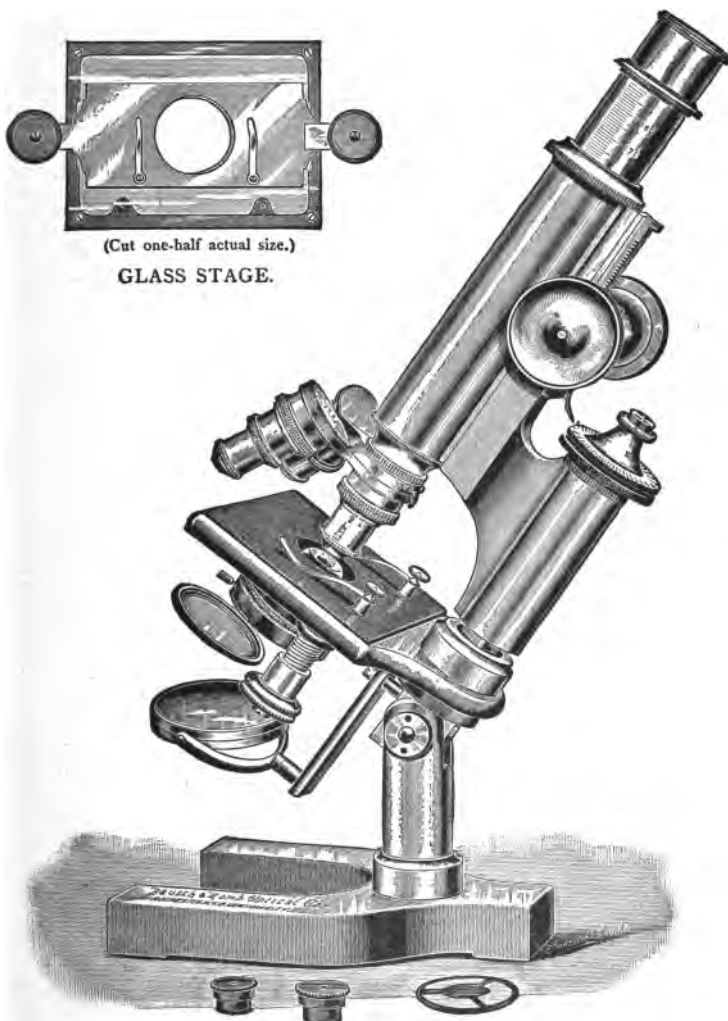


FIG. 5. — Lens of Crown and Flint Glass.

lens. One is crown glass, or silicate of potassium and lime, which has an index of refraction of about 1.52 and a dispersive power of 0.036; the other glass is flint, or silicate of potassium and lead, with an index of

refraction of about 1.63 and a dispersive power of 0.059. These two glasses when combined have the power of so acting on each other that when a ray passes through them it is not only refracted but the decomposition of light produced by one glass is considerably overcome by the other, and the colors are again recombined into white light and brought to a focus with more or less distinctness in the image. The angles of these lenses must be unequal, in order that the dispersive powers may be the same. The usual form for the crown glass is bi-convex and that for flint is plano-convex. They are cemented together



(Cut one-half actual size.)

GLASS STAGE.

(Cut one-half actual size.)

BB 8—CONTINENTAL MICROSCOPE.

PLATE II.

with Canada balsam. A lens so constructed is called *achromatic*.

For a long time it was found to be impossible with the information possessed by the opticians, entirely to overcome chromatic aberration, even after many experiments in this country and abroad had produced numerous different kinds of glasses of the flint and crown varieties.

Some years ago Professor Abbe and Dr. Schott, two skilful experimenters of Jena, Germany, began investigations with different compounds of vitreous materials for the purpose of discovering some method for correcting chromatic aberration and other defects which were so troublesome to the optical manufacturers. They succeeded in discovering, by means of elaborate and expensive experiments, compounds closely resembling glass, whose properties are so remarkable that a lens manufactured from the compound for the microscope gives an image with marvellous distinctness, even to the outer limits of the field. These glasses do not possess silica in their composition, which we found to be the case with the flint and crown glasses in the achromatic lenses above described, but in the Jena glass the silicon is replaced by boron in the flint series and by phosphorus in the crown series. Since 1889 the Zeiss Company of Jena, Germany, have been making objectives, eyepieces, and other lenses from this new glass, and have supplied other optical companies in different parts of the world with the compound, so that now the best microscopes are furnished with objectives made of the new composition. The Zeiss Company, which have the patent rights on the Abbe and Schott discoveries, call the glasses made from the new compound *apochromatic*.

This discovery has produced material of certain refractive and dispersive properties which make the glass specially valuable for objectives, and the amount of light concentrated in the image is greatly increased over that yielded by lenses made of flint and crown glass. The apochromatic objectives made of this new glass are much more expensive than the ordinary achro-

matic objectives, and they require in their manufacture greater skill. It is quite desirable to have the laboratory equipped with these apochromatic objectives, and some delicate work demands their use; still much of the work required of students can be well performed with microscopes supplied with achromatic objectives, provided they are carefully made by the best manufacturers of microscopes.

The compound microscope.

— Figure 6 is a longitudinal section of the instrument and shows that it consists of two tubes, one sliding in the other, a stage and stand, besides the lenses, called *oculars* and *objectives*. The inner tube carries in the upper end the ocular *OC*, and to the lower end of the outside tube is screwed the objective, *B*. The tubes are blackened on the inside so that all reflection of the light rays, after passing through the objective, will be obviated. The rays of light com-

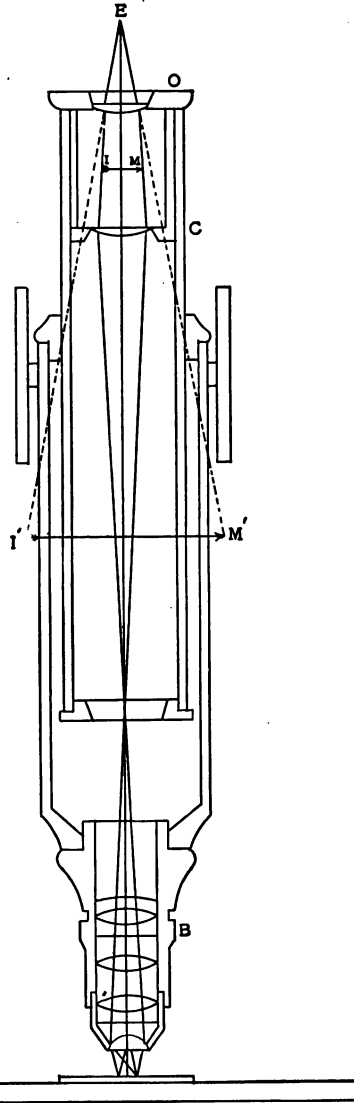


FIG. 6. — Section of Compound Microscope.

ing from the object, mounted on the glass slide *S*, pass through the objective *B* to eyepiece *OC* and are caught by the eye situated at *E*. The real image is formed at *IM* and the virtual image at *I'M'*. This virtual image is projected from the eye. The sliding of the tubes, one in the other, permits of limited elongation, so that the length may be adapted to the character of the objective in use. For instance, the objective made by a German optician will not produce satisfactory results when used on an American stand, unless especially adjusted for it. The inner or draw-tube is usually graduated into inches or millimeters to enable the microscopist to secure a known length at will. At the lower end of the draw-tube is a screw thread to hold the auxiliary objective when the *apertometer* is in use, determining the numerical and angular aperture of the objectives. The objectives made by Zeiss require a length of tube of 160 mm., and this length is "reckoned from the contact surface of the objective thread to the upper end of the body on which the eyepiece rests."

The following table is given to show how much this matter of tube-length varies with different manufacturers throughout the world:—

Reichert of Vienna	160 to 180	mm.
Swift & Son of London	165 to 228½	"
Zeiss of Jena, Germany	160	"
Winkel of Göttingen	220	"
Ross of London	254	"
Leitz of Wetzlar, Germany	170	"
R. & J. Beck of London	254	"
Grunow of New York	203	"
Bausch & Lomb of Rochester, N.Y.	160 to 216	"
Spencer & Co. of Buffalo, N.Y.	160 to 235	"

The thickness of the cover-glass, on slide *S* (Fig. 6), has a great influence also over the results secured with the objective, because the light is changed in its character and direction after passing through the cover-glass, and many rays will be lost unless the proper adjustment is made in the objective. It is

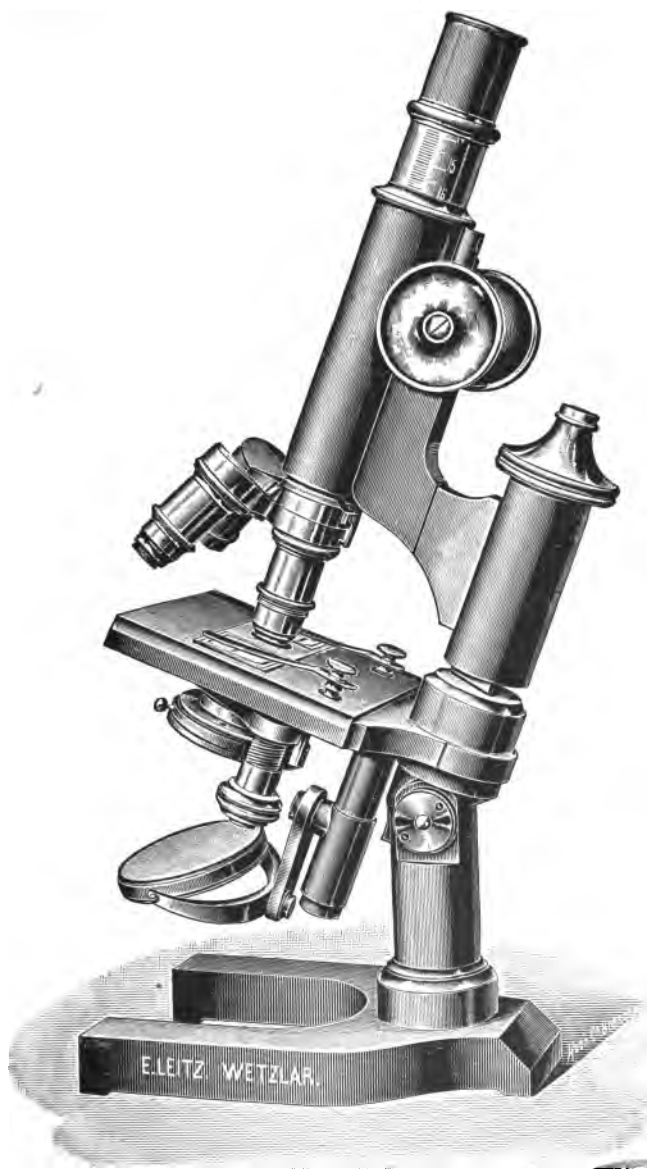


PLATE III.—LEITZ MICROSCOPE.

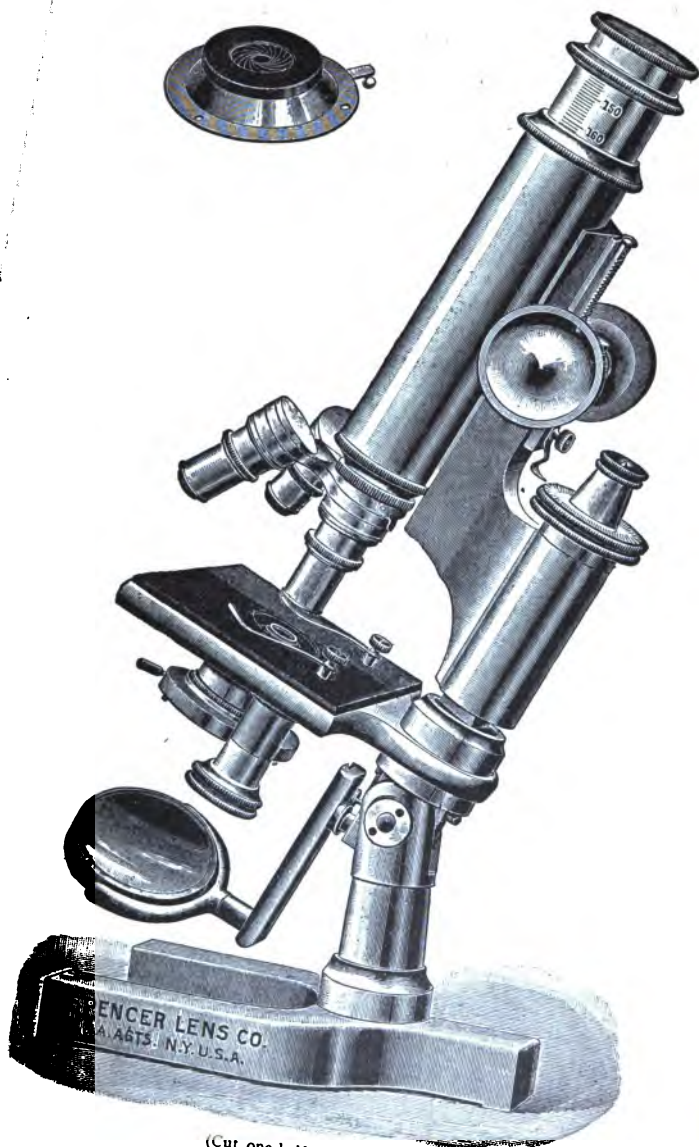


best, therefore, to use a cover-glass gauge, when one of the higher series of objectives is on the microscope, and mount with cover-glasses of an estimated thickness.

Another way of determining the thickness of the cover-glass is recommended by Zeiss as follows: "The divisions on the milled head of the micrometer-screw of Stands Ia-IV (see Frontispiece) furnish a means for exactly registering the vertical movements of the tube. In our present stands each division corresponds to an elevation or depression of the tube in the direction of the optical axis of 0.01 mm., the half-divisions representing 0.005 mm. By this means measurements of thickness may be made with a considerable degree of accuracy. The upper and lower surfaces of the object are successively focussed, and the amount read off on the milled head by the fixed index. In doing this, care must be taken to make both adjustments by a rotation of the screw in the same direction. The thickness of an object in air is then equal to the difference between the two readings.

"Similarly, the thickness of any other substance may be measured by this method. The estimation of the thickness of a cover-glass, for instance, is best done as follows: with a medium-power dry lens and eyepiece, using central illumination, focus the upper and lower surfaces of the cover-glasses of known thickness — e.g. the covers of an Abbe test-plate — and note the apparent thickness so obtained. A comparison of this with the known true value gives, once for all, the coefficient for reducing to their true values measurements of any other covers, made with the same objective under precisely similar conditions. Roughly speaking, this coefficient is equal to 1.52 (the refractive index of glass). The thickness of a section is estimated in a similar manner."

Besides the lengthening of the tube, above indicated, the instrument is also provided with an adjustment which permits the raising and lowering of the entire tube-system and thus bringing the object into focus. This movement is under the control of two mechanisms: one called the *coarse adjustment* and the other



(Cut one-half actual size.)
CONTINENTAL FORM NO. 1.
PLATE IV.

the *fine adjustment*. The first is made by means of rack and pinion movement. The second is accomplished by turning the milled-head micrometer-screw, which is generally graduated.

In the cheaper forms of instruments the rack and pinion are wanting and the coarse adjustment is made by sliding the tube-system up and down by a twisting motion with the thumb and finger.

The stand.—The base of the stand should be made of strong material, and should maintain a firm and steady position even when the tube of the instrument is inclined at any angle. The Continental, and some of the American stands also, have bases in the form of horseshoes, while many of the other makes consist of a three-pronged base.

The stage occupies a position on the microscope just underneath the tube and is firmly fixed to the stand. In its most simple form it consists of a metal table, sometimes rectangular and at other times round, with a hole through the centre for the passage of light from the illuminating system beneath. On the stage are two spring-clips to be used in holding the slide well clamped to the stage. Diaphragms are fitted to the opening in the stage to control the quantity of light.

The mechanical stage placed on top of the simple stage contains the mechanism necessary for producing the required movements to bring all portions of the object readily into view. "The available lateral movement measures 50 mm., and the stage moves through 35 mm. in the direction from front to back. The amounts are read by vernier and scales. The mechanical stage (see Frontispiece) is so solidly constructed that possessors of a stand fitted with it may well dispense with the plain vulcanite stage. The mechanism (*LK*) which serves for the lateral movement of the object lifts off after unscrewing the fixing screw *L*, whereby the solid lower part of the stage becomes free. By means of the milled head *W* this lower part of the stage can be made to move in a forward and backward or—if the whole stage be rotated—in any other desired direction."

How to use the mechanical stage.—The Bausch & Lomb Optical Company in this country and the Zeiss Company in Germany manufacture the best form of mechanical stage. The Zeiss pattern is shown in the Frontispiece. This stage permits of several movements in making measurements of objects. The milled-head screw *K* gives a movement from right to left, and *W* turns the stage in a direction at right angles to *K*. There are two millimeter scales on the stage; one is shown in the illustration just under the milled-head screw *K*, and the other is to the extreme left of the stage and not visible in the illustration. One is at right angles to the other, thus allowing measurements in two directions. A vernier slides on each of these scales, thus giving a means for delicate measurements. The screw *L* is used for firmly clamping the stage. By the use of this mechanical stage lengths and breadths can be measured with great degree of accuracy.

The illuminating apparatus.—This is an important part of the microscope and should be well and accurately made. It is situated beneath the stage. The simplest form is a circular mirror with two reflecting surfaces, and so mounted as to allow of the movement necessary to catch the light from any direction and reflect it through the stage upon the object. The diameter ought to be at least 2 inches, so that an ample amount of light can be reflected by it.

There are two reflecting surfaces, one plane and the other concave. The first is adapted to the use of lower power-objectives, because the light is reflected with initial intensity. The concave mirror concentrates the rays and gives a brighter surface on the stage than that produced by the plane surface, and it is used with the higher powers, except when the condenser is in place. The points to be observed by the student in securing the best results with the reflector are:—

1. Central illumination.
2. A uniform and even illumination over the entire field.
3. Not too intense light.

The control of the quantity of light may be had by the judicious use of diaphragms, the rule being to reflect only enough light clearly to define the object and avoid straining the eyes.

How to use the condenser. — Professor Abbe has probably done more to develop this important part of the microscope than any

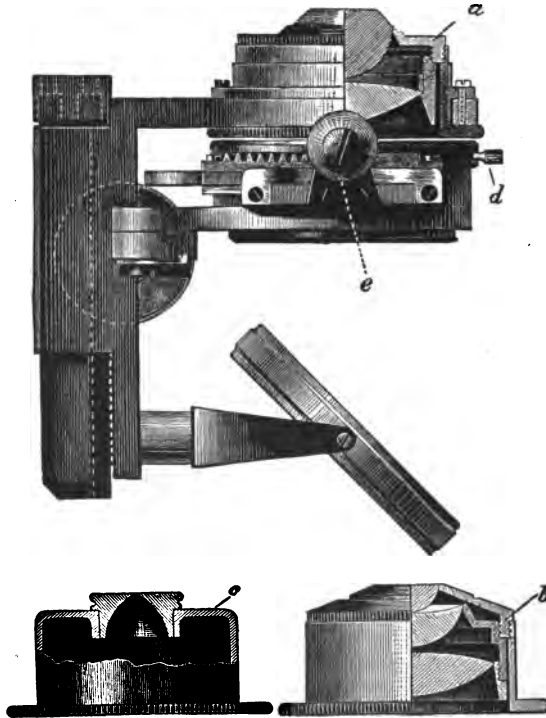


FIG. 7. — Abbe's Illuminating Apparatus.

other party. The essential features of the Abbe condenser are to collect the rays from the reflector in a system of short focus and concentrate them in an illuminated cone of large aperture which has its focal point in the plane where the object is situated. This condenser consists of (1) the cylinder for carrying the iris diaphragm and (2) the swing-out condenser.

The use of this instrument must be governed entirely by the character of objects under examination. The full aperture of the illuminating cone is only adapted to objects deeply stained and of fine granular condition in connection with objectives of large aperture. In all other cases the condensers must be stopped down.

This instrument will also permit of dark ground illumination by moving the diaphragm eccentrically with the rack and pinion attached to the carrier, thus cutting off the central rays and producing oblique illumination.

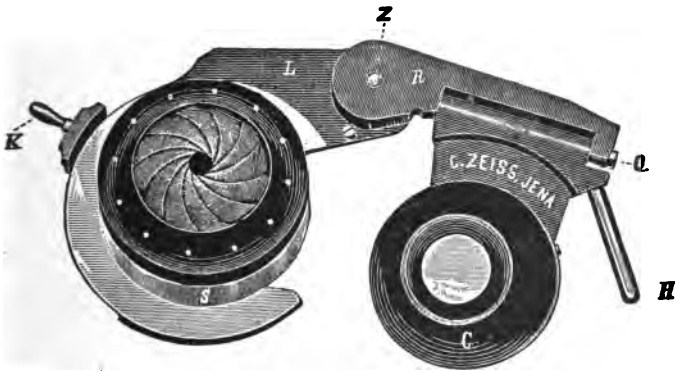


FIG. 8. — Zeiss' Swing-out Condenser.

The condenser is so mounted that the system may be made to approach or recede from the stage, and in this way the degree of illumination is kept under perfect control. Between the condenser and the reflector is an iris diaphragm, which is very essential to the system in reducing or enlarging the cone of light. This diaphragm is controlled by means of a pin *d* (Fig. 7). In this illustration, *a* is the condenser system of 1.20 numerical aperture; *b* is the condenser system of 1.40 numerical aperture; *c* is the cylinder diaphragm; and *e* is the milled head for throwing the diaphragm out of centre. The "swing-out condenser" is shown more in detail in Fig. 8. It is thus described by the manufacturer: "The condenser system is by a suitable

mechanism so connected to the sleeve enveloping it that, after swinging the diaphragm carrier *D* aside (toward the right of the observer), it may by means of the lever *H* projecting from under the stage be swung downward about the axis *Q* and to the left about the pivot *Z*. When the condenser is not being used, the width of the illuminating cone is regulated by means of

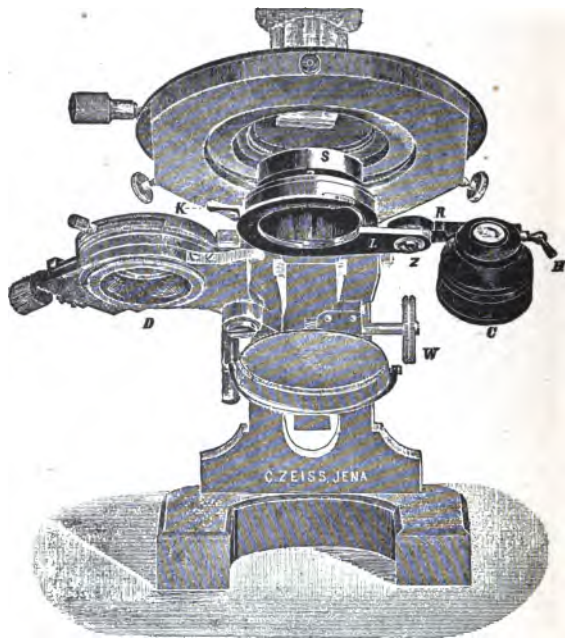


FIG. 9.—Swing-out Condenser attached to Microscope.

the *iris cylinder diaphragm*, which is permanently attached to the apparatus and is actuated by the small pin *K* seen on the right. The iris diaphragm is so shaped that the edge of its smallest opening closely approaches the object slide."

The iris diaphragm in the sub-stage is the best contrivance for controlling the light of any diaphragm now known. There are many advantages in its use, among which may be mentioned

the time and inconvenience saved in substituting one metal-disk for another, as is required in the old system ; the gradual exclusion of the rays as the iris is contracted, thus producing marked effects in the appearance of the object.

The revolving nose-piece. — This is a mechanical device for enabling the manipulator to rapidly and conveniently change from one objective to another. The nose-piece (Fig. 10) is screwed to the lower end of the tube and is so con-



FIG. 10.—Revolving Nose-piece.

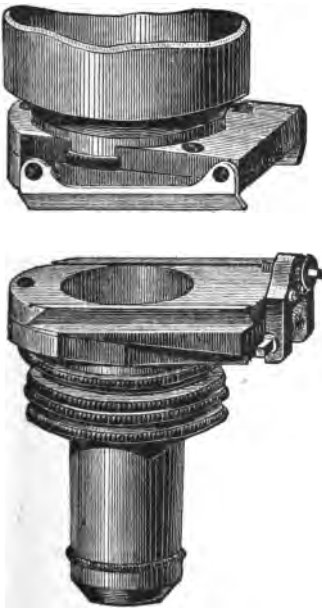


FIG. 11.

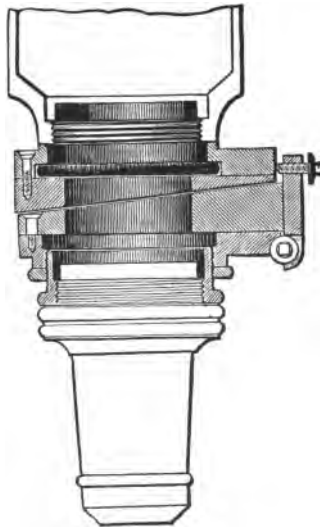


FIG. 12.

Zeiss' Sliding Objective Changers.

structed as to hold two, three, or four objectives at the same time. By revolving the instrument each objective is immediately brought into use, requiring but little adjustment of the microscope to bring out the image clear and distinct.

Sliding objective changers. — Dr. Roderick Zeiss, of Germany, has devised an apparatus (Figs. 11 and 12) which is considered by most microscopists to be superior to the revolving nose-piece. This instrument is called the *sliding objective changer*. The appliance consists of two parts: (1) *the tube-slide*; (2) *the objective slide*. The first is permanently screwed to the lower end of the tube, in the same manner explained with the nose-piece. The lower part of the tube-slide contains a grooved plane inclined to the optic axis of the microscope. The objective slide contains a similar grooved plane inclined also to the optic axis, and when the objective is pushed into working position the inclined planes permit the lens to approach gradually toward the slide containing the object without danger of breaking the glasses. This sliding objective changer is adapted and corrected to fit certain objectives and microscopes, but it may be altered to suit any standard microscope and lens by simply adjusting the screws, which move at right angles to each other so that centring may be accomplished in either direction.

CHAPTER II

OCULARS AND OBJECTIVES

The oculars. — There are two kinds of oculars, or eyepieces, which are termed *positive* and *negative*. The first comprises those glasses in which the real inverted image is made outside the ocular, while the latter have the image produced within the glass. The positive eyepiece is a simple magnifier and may be used in place of the ordinary hand-glass; the negative lens cannot be so used.

The most important oculars are : —

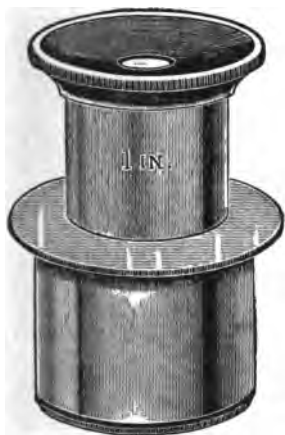


FIG. 13. — Huyghenian Ocular. American Pattern.

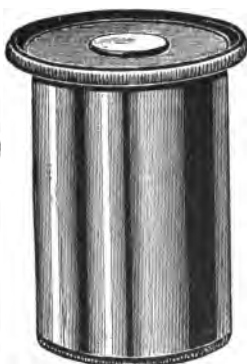


FIG. 14. — Huyghenian Ocular. Continental Pattern.

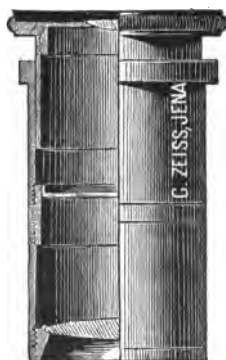


FIG. 15. — Section of Ocular.

1. *Huyghenian oculars* (Figs. 13 and 15). — These consist of two plano-convex lenses, one at each end of the brass tube. The upper lens has its plane surface toward the observer, and the lower lens has its convex surface toward the object. The upper is called the *eye-glass*, and the lower is known as the *field* or *collecting glass*. Between these lenses is placed a diaphragm

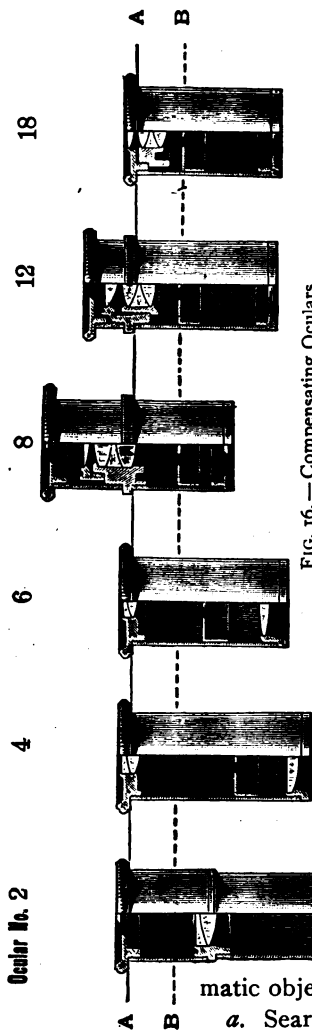


FIG. 16.—Compensating Oculars.
A-A, Plane of Upper Edge of Tube. (Zeiss.) B-B, Lower Focal Plane of the Eyepiece.

near the focus of the eye-glass. This ocular belongs to the negative type and is used in most microscopes made on the American and Continental plans. There are two kinds, viz.:—

a. The type which is generally used on American microscopes (Fig. 13).

b. The Continental form, which is used on all German and French instruments (Fig. 15).

2. *Ramsden's positive ocular.*—These eyepieces do not distort the image as much as is the case with the Huyghenian oculars, and they are, therefore, more suitable for micrometer measurements. They consist of two plano-convex lenses, with the convex surface turned toward each other.

3. *Compensating oculars.*—The eyepieces by this name are manufactured by Zeiss, and are adjusted for use with the apochro-

matic objectives. Two classes are made:—

a. Searching oculars, which are used for reducing to the lowest possible degree the magnification produced by the objective and facilitating the location of the image.

b. Working eyepieces. These are also the results of the inventive genius of Zeiss, and were designed especially to be used in conjunction with the apochromatic objectives made by the same house. They are numbered 1, 2, 4, 6, 8, 12, and 18. "The number which denotes how many times an eyepiece, when used at a given tube-length, increases the initial magnifying power of the objective affords the measure of the eyepiece magnification and, at the same time, furnishes the figures for their rational numeration. On this basis the series of our compensating eye-pieces is arranged according to their magnifying powers. The magnification obtained by combining a compensating eyepiece with any apochromatic objective is found by multiplying its number by the initial magnification of the objective. An objective of 3.0 mm. focus, for example, yields in itself a magnification of 83.3 (calculated for the conventional distance of vision of 250 mm.); eyepiece 12 therefore gives with this objective a magnification of $12 \times 83.3 = 1000$ diameters." (ZEISS.)

These compensating oculars are corrected for aberration in the extra-axial portions of the objectives. It has been well known that all objectives of considerable aperture produce color rays on the margin of the visual field, even though the central rays may come through the lens free from confusion. This difficulty has been overcome by the use of oculars which have been made to possess an equivalent error but of the opposite character, or in other words the image formed by the red ray is larger than that formed by the blue, and in this manner the eyepieces are made to correct the unequal magnification of different colors, and the resulting image is free from all color and confusion.

"The mounts of the eyepieces are such that the lower focal points of all the numbers of each series lie in the same plane when the eyepieces are inserted in the body-tube. No alteration of focus is therefore required on changing the eyepiece, and the optical tube, *i.e.* the distance between the upper focal point of the objective and the lower of the eyepiece, which is the determining element of the magnifying power, remains constant. In the Continental microscopes this tube-length is 180 mm., irrespec-

tive of small differences between the various objectives, the length of the body, from the screw-collar of the objective to the upper of the tube on which the eyepieces rest, being 160 mm.

"The numbering of these eyepieces is carried out on the plan proposed by Professor Abbe. Accordingly, the number which denotes how many times an eyepiece, when used with a given tube-length, increases the initial magnifying power of the objective, affords a correct measure of the eyepiece magnification, and at the same time furnishes the figures for their rational numeration. In this way our compensating eyepieces are numbered according to their magnifying powers, viz.: 2, 4, 6, 8, 12, 18, 27.

"The magnification obtained by the combination of a compensating eyepiece with any apochromatic objective is obtained by multiplying its number by the initial magnification of the objective as given in the following table:—

	Numerical Aperture	Equivalent focus in mm.	Initial Magnification
Dry Series	0.30	24.0 16.0	10.5 15.5
	0.65	12.0 8.0	21 31
	0.95	6.0 4.0 3.0	42 63 83
Water Immersion	1.25	2.5	100
Homogeneous Immersion	1.30	3.0 2.0 1.5	83 125 167
	1.40	3.0 2.0	83 125

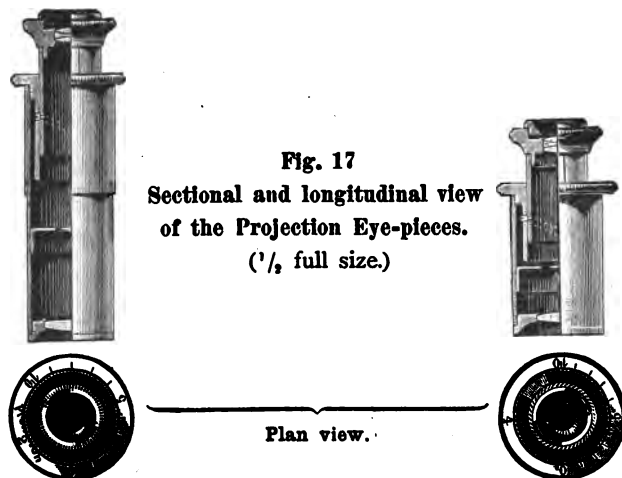
An objective of 3.0 mm. focus, for example, yields in itself a magnification of 83.3 (calculated for the conventional distance of vision of 250 mm.); eyepiece 12 in conjunction with this objective gives therefore a magnification of $12 \times 83.3 = 1000$ diameters." (ZEISS.)

The Bausch & Lomb Optical Company of this country have followed the Zeiss Company in making a set of compensating oculars to be used with their series of apochromatic objectives. These oculars, like the Zeiss pattern, have chromatic error opposite to that of the objective, and these two lenses acting on each other eliminate the chromatic error and produce images which are clear and free from color. The oculars are numbered 2, 4, 8, 12, 16. The number 2 is used as a finder and for photography; 4 and 8 are for general work; 12 is called into use when the utmost power of the objective is required; and 16 is intended for aid in securing the finest adjustment in the objective.

The magnification can be obtained by "dividing 250 mm., the distance for distinct vision, by the focal length of the objective and multiplying the quotient by the number of the ocular; thus the lowest power of the series is equal to $(250 \text{ mm.} \div 38) \times 2 = 13$, and the highest to $(250 \text{ mm.} \div 3.5) \times 16 = 1136$." The tube-length used with these objectives and compensating oculars is the standard length of 160 mm.

4. *Projection oculars.*—These eyepieces are used for projecting an image on a screen or for photographic purposes in making photo-micrographs. They are corrected so that they may be used with the apochromatic objectives, and the magnification is determined in the same manner as in the case of the compensating oculars. The magnification of the image on the screen, or ground glass of the camera, may be determined by dividing the distance between the ocular and the image by the focal length of the objective and multiplying the result by the number stamped on the ocular. The distance must be expressed in millimeters, because the other factors of the equation are so expressed. This will hold good for long distances, but for short spaces the results are not so accurate.

The field is limited by the diaphragm between the two systems of lenses. The projection lens is so mounted that it may be moved from or toward the diaphragm. Before beginning photographic operations, it is best to focus on the screen, or ground glass, by extending or pushing into the tube the projection-glass until the outlines of the diaphragm are distinctly marked, and



then the apparatus is adjusted for work. If the microscope is near the screen, it will be necessary to extend the projection-glass from the diaphragm.

In the use of the projection ocular care must be exercised in adapting it to the objectives. The oculars made by Zeiss are numbered 2, 4, 3, and 6. The 2 and 4 systems are adjusted for the Continental tubes, or 160 mm., and they have an amplification of 2 and 4 times. The 3 and 6 oculars are to be used with the English tube of 250 mm. They are specially designed for the apochromatic objectives and should only be used with them when results of the highest order are required. In their use also care should be exercised that the length of the tube is fixed at that figure for which the oculars are manufactured.

The images produced by these eyepieces are remarkably clear and accurate, and the photographs made by them are exact reproductions of the images seen by the observer through the ordinary ocular.

5. *Micrometer ocular.* — This is an eyepiece made according to the Huyghenian pattern with the eye-lens adjustable so that a focus may be secured on the measurements of the micrometer resting on the diaphragm between the eye-lens and the field-glass. Figure 14 gives a correct idea of this ocular. The micrometer that accompanies this instrument is shown in Fig. 18, which consists of a disk of

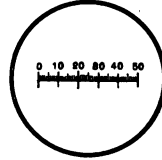


FIG. 18. — Ocular Micrometer.

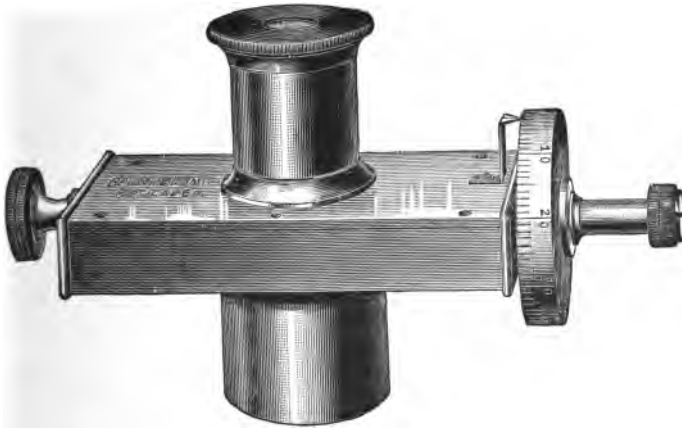
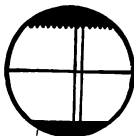


FIG. 19. — Filar Micrometer.

glass divided into arbitrary lines which, when compared with the stage micrometer, will give the size of the image produced by the object. It is best to construct a table for each objective, with the assistance of the stage micrometer, so that the values of the divisions on the eyepiece micrometer may be quickly determined.



Field of Filar Micrometer.

6. *Filar micrometer.* — This instrument consists of an ocular with cross-hairs so arranged that

they can be moved by a micrometer screw across the field of observation. The milled-head screw on the left controls one of the vertical hairs. The graduations on the circumference on the right of the figure give the value of the motion of the micrometer screw; the reading is to 0.005 mm. The horizontal and one vertical hair are stationary, while the other vertical hair is moved back and forth across the field. To measure with this instrument a stage micrometer must be used in connection with it.

If one of the compensating oculars is used with this micrometer in connection with the apochromatic objectives, it will not be necessary to construct the table of magnification, since the enlargement can be read off at once without calculation, because the divisions in the ocular have been carefully estimated with a known tube-length and with each apochromatic objective, so that each division on the micrometer represents as many micra (0.001 mm. μ^1) as there are millimeters in the focal length of the objective. For instance, if the Zeiss series of objectives and ocular 6 are used, the magnification will be as follows:—

Apochromatic objectives	16 mm.	8 mm.	4 mm.	3 mm.	2.5 mm.	2 mm.	1.5 mm.
Value of intervals	16 μ	8 μ	4 μ	3 μ	2.5 μ	2 μ	1.5 μ

Thickness of cover-glasses. — With the use of the high-power objectives it becomes absolutely necessary that the thickness of the cover-glass, which protects the section, should be known. This is essentially important because of the refraction of the light produced by the cover-glass, and the influence the glass has over the residue of chromatic and spherical aberration found in nearly all objectives. Although this influence is inappreciable in the low-power objectives, still, when the immersion lenses are used, this factor becomes very important, and the thickness of the cover-glass must be known and the correction allowed for it in the adjustment of the objective and in the length of the tube.

¹The unit of measure is called "*micron*" — which is .001 of a millimeter. This measure was first suggested in 1859 by Harting, and in 1869 Listing proposed that the Greek letter μ be used to designate the .001. And 16 μ would therefore mean .001 $\times$ 16 or .016 millimeter, or 16 microns.

Several excellent instruments have been made by manufacturers for measuring the thickness of cover-glasses, and the two illustrated in Figs. 20 and 21 give first-class results. Figure 20 is made by the Bausch & Lomb Optical Company, and is used by placing the cover-glass in the opening between the

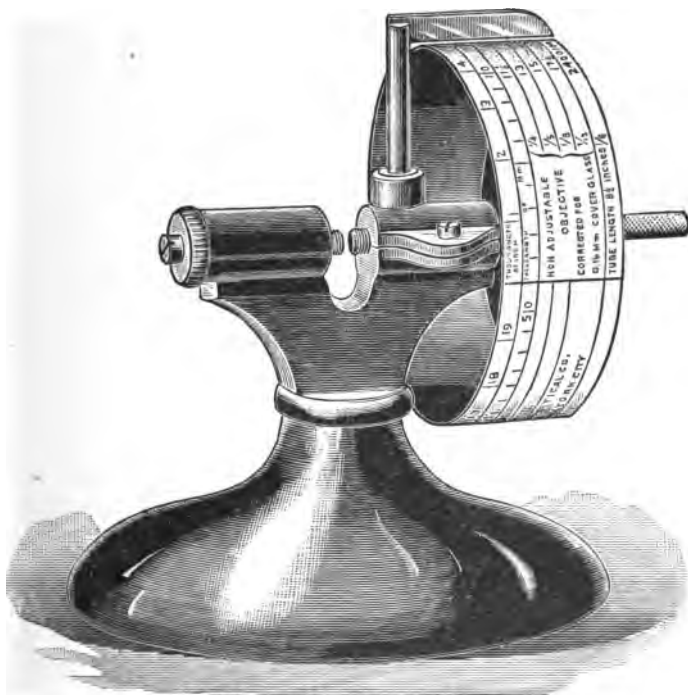


FIG. 20. — Cover-glass Gauge. (B. & L.)

screws passing through the axis of the instrument. This screw is termed the micrometer-screw. By turning the milled head at the right of the instrument the two screws are brought into contact with the glass, and the thickness is read off under the knife-edge on top of the cylinder. The divisions are in thousandths of an inch and in hundredths of a millimeter. The scale also gives the exact tube-length to be used for each cover-glass measured.

Figure 21 is manufactured by the Zeiss Company, and the measurement is effected by pushing the lever to one side, inserting the cover-glass between the jaws of the clip projecting from one side of the gauge, and then reading the thickness in milli-

meters or inches by means of the pointer on the disk.



FIG. 21. — Cover-glass Gauge. (Zeiss.)

The objective. —

This is the most important part of the microscope; the material of which it is made must be of the best, and its manufacture must

be by the most skilful opticians. Great skill has been developed in America and abroad in calculating the lenses for objectives, and workmen of remarkable expertness have been trained in their manufacture.

In the selection, therefore, of a microscope the student with limited means should purchase a good stand with few, if any, accessories on the stage, and put most of his money in one or two of the best objectives, of comparative low power, to be found in the market. As his work advances in interest, and his income will permit, he may add gradually to his list of objectives, so that in the course of time he will possess an outfit with which he will be able to execute first-class work of a most delicate character.

Objectives for compound microscopes are all achromatic. That is to say, they are so constructed that the colored rays decomposed out of the white light by the first lens are recomposed by the next lens into white light and the image is produced clear and sharp. When the *three* principal rays of the spectrum are united by the lens system of the objective in one point of the axis and the correction of *two* different colors has been at the same time accomplished, such an objective is called

apochromatic. These are now manufactured in Germany and in America, and they are the finest, most expensive, and most satisfactory objectives to be obtained in the world. The image made by them is distinct and sharp to the outer limits of the field, and the results seem to be most perfect in all respects.

Objectives are generally divided into two classes :—

1. Dry objectives, and
2. Immersion objectives.

The first, or dry series, are used for examining objects in air ; that is to say, there is no liquid interposed between the lens and the slide.

The second class requires the interposition of a drop of water or oil between the lens and the slide-cover, to catch those rays

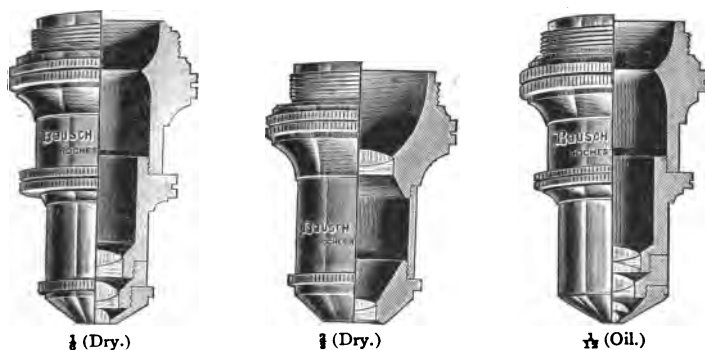


FIG. 22. — Dry and Oil Immersion Objectives. (B. & L.)

of light which are considerably refracted by the cover-glass, and which, without the aid of the liquid, would pass beyond the reach of the objective.

The immersion objectives constructed for oil are called *homogeneous*. Cedar oil is usually found best adapted for these glasses. This oil has the same index of refraction as crown glass, viz. : 1.515. The index of refraction of water (1.336) being lower than oil, some of the rays are not caught by it, and hence it is not so useful as an immersion medium. The homogeneous

objectives have a greater frontal distance and give clearer images than are possible with dry objectives.

In the manufacture of objectives by different houses in this country and abroad, the shape, size, and screw-threads of the lens-mounts were almost as numerous in variety as was the number of manufacturers. But as the demand for delicate and multiplied work with the microscope increased in different

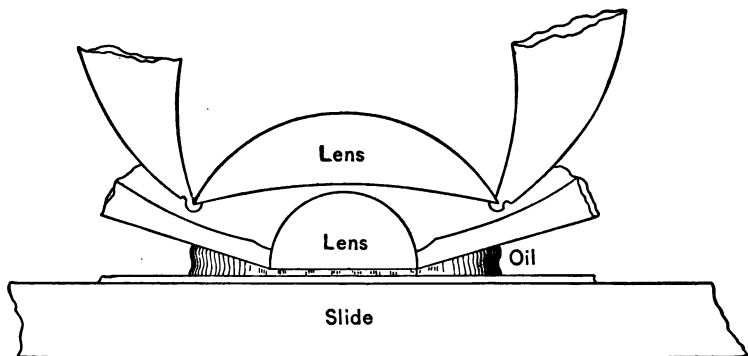


FIG. 23.—Section of Oil Immersion Objective showing Position of Oil.

parts of the world, came also the demand, on the part of the microscopists, for greater facilities in handling the instrument. It was then that the manufacturers realized the importance of making certain parts of the instrument, made at one factory, interchangeable with similar portions made by another firm.

The most important part of the microscope subjected to this evolution was the screw-thread used in attaching the objective to the tube of the instrument. Even in this late day we have the *German thread*, the *English thread*, and the *American thread*. Some years since, however, the Royal Microscopical Society of England devised a screw which is now called "*The Society's Screw*," and most of the opticians in America and abroad have adopted it, so that now stands purchased in one country may receive objectives made in another portion of the world.

Objectives were formerly designated by letters, but the almost universal custom now is to mark them with numbers indicating

the focal distance of the lens. And it would also be of great advantage if all objectives contained figures to designate the *angular aperture*, *i.e.* the angle formed by the two most divergent rays of light emanating from the axial point of the object and entering the objective. Professor Abbe found that it was difficult to satisfactorily compare all objectives by the use of the angular aperture, since some lenses were dry, some water, and others were homogeneous or oil-immersion objectives; and it was found necessary, therefore, to take into consideration the refractive index of the medium intervening between the objective and the cover-glass, so he introduced in 1873 the method which he termed

Numerical aperture (N.A.). — This represents the capacity of the lens for receiving the rays of light from the object and transmitting them to the image. "The ratio between the radius of the emerging pencils measured in the upper focal plane of the equivalent focal length of the latter." We may express this ratio by the following formula:—

$$\text{N.A.} = n \sin u,$$

in which n designates the index of refraction of the medium between the object or cover-glass, and $\sin u$ is the sine of half the angle of aperture.

By means of this formula we can now accurately compare an objective of one class with that of another class. For example, if we take a dry objective, the medium of which is air, and apply it to our formula, we will get 1 for the value of n , by the use of a table of natural sines; for the immersion lenses the value of n will vary according to the character of the media used between the objective and the cover-glass, governed of course by the value of the refractive index of the medium used in each case. To illustrate the use of this formula, let us take one example in the case of the oil immersion objective. The refractive index of cedar oil is 1.52, the angle of aperture of the objective is, say, 60° , half this angle is 30° . From the table of natural sines we get for 30° 0.50; applying to the formula we have

$$\text{N.A.} = 1.52 \times 0.50 = 0.76.$$

APERTURE TABLE

[Copied from *Journal of the Royal Microscopical Society*]

Numerical Aperture ($n \sin u = a$)	Correspond'g Angle ($2u$) for			Limit of Resolving Power in Lines to an Inch			Illuminating Power (a^2)	Pene- trating Power ($\frac{1}{a}$)
	Air ($n=1.00$)	Water ($n=1.33$)	Homogeneous Immersion ($n=1.52$)	White Light ($\lambda=0.5269 \mu$, Line E)	Monochro- matic (Blue) Light ($\lambda=0.4861 \mu$, Line F)	Photog- raphy ($\lambda=0.4000 \mu$, Near Line h)		
1.52	—	—	180° 0'	146,543	158,845	193,037	2.310	.658
1.51	—	—	166° 51'	145,579	157,800	191,767	2.280	.662
1.50	—	—	161° 23'	144,615	156,755	190,497	2.250	.667
1.49	—	—	157° 12'	143,651	155,710	189,227	2.220	.671
1.48	—	—	153° 39'	142,687	154,665	187,957	2.190	.676
1.47	—	—	150° 32'	141,723	153,620	186,687	2.161	.680
1.46	—	—	147° 42'	140,759	152,575	185,417	2.132	.685
1.45	—	—	145° 6'	139,795	151,530	184,147	2.103	.690
1.44	—	—	142° 39'	138,830	150,485	182,877	2.074	.694
1.43	—	—	140° 22'	137,866	149,440	181,607	2.045	.694
1.42	—	—	138° 12'	136,902	148,395	180,337	2.016	.709
1.41	—	—	136° 8'	135,938	147,350	179,067	1.988	.709
1.40	—	—	134° 10'	134,974	146,305	177,797	1.960	.714
1.39	—	—	132° 16'	134,010	145,260	176,527	1.932	.719
1.38	—	—	130° 26'	133,046	144,215	175,257	1.904	.725
1.37	—	—	128° 40'	132,082	143,170	173,987	1.877	.729
1.36	—	—	126° 58'	131,118	142,125	172,717	1.850	.735
1.35	—	—	125° 18'	130,154	141,080	171,447	1.823	.741
1.34	—	—	123° 40'	129,189	140,035	170,177	1.796	.746
1.33	—	180° 0'	122° 6'	128,225	138,989	168,907	1.769	.752
1.32	—	165° 56'	120° 33'	127,261	137,944	167,637	1.742	.758
1.30	—	155° 38'	117° 35'	125,333	135,854	165,097	1.690	.769
1.28	—	148° 42'	114° 44'	123,405	133,764	162,557	1.638	.781
1.26	—	142° 39'	111° 59'	121,477	131,674	160,017	1.588	.794
1.24	—	137° 36'	109° 20'	119,548	129,584	157,477	1.538	.806
1.22	—	133° 4'	106° 45'	117,620	127,494	154,937	1.488	.820
1.20	—	128° 55'	104° 15'	115,692	125,404	152,397	1.440	.833
1.18	—	125° 3'	101° 50'	113,764	123,314	149,857	1.392	.847
1.16	—	121° 26'	99° 29'	111,835	121,224	147,317	1.346	.862
1.14	—	118° 0'	97° 11'	109,907	119,134	144,777	1.300	.877
1.12	—	114° 44'	94° 55'	107,979	117,044	142,237	1.254	.893
1.10	—	111° 36'	92° 43'	106,051	114,954	139,698	1.210	.909
1.08	—	108° 36'	90° 34'	104,123	112,864	137,158	1.166	.926
1.06	—	105° 42'	88° 27'	102,195	110,774	134,618	1.124	.943
1.04	—	102° 53'	86° 21'	100,266	108,684	132,078	1.082	.962
1.02	—	100° 10'	84° 18'	98,338	106,593	129,538	1.040	.980
1.00	180° 0'	97° 31'	82° 17'	96,410	104,503	126,998	1.000	1.000
0.98	157° 2'	94° 56'	80° 17'	94,482	102,413	124,458	.960	1.020
0.96	147° 29'	92° 24'	78° 20'	92,554	100,323	121,918	.922	1.042
0.94	140° 6'	89° 56'	76° 24'	90,625	98,223	119,378	.884	1.064

APERTURE TABLE (Continued)

[Copied from *Journal of the Royal Microscopical Society*]

Numerical Aperture ($n \sin u = a$)	Correspond'g Angle ($2u$) for			Limit of Resolving Power in Lines to an Inch			Illuminating Power ($\frac{1}{a^2}$)	Penetrating Power ($\frac{1}{a}$)
	<i>Air</i> ($n=1.00$)	<i>Water</i> ($n=1.33$)	<i>Homogeneous Immersion</i> ($n=1.52$)	White Light ($\lambda=0.5269 \mu$, Line E)	Monochromatic (Blue) Light ($\lambda=0.4861 \mu$, Line F)	Photography ($\lambda=0.4000 \mu$, Near Line A)		
0.92	133° 51'	87° 32'	74° 30'	88,697	96,143	116,838	.846	1.087
0.90	128° 19'	85° 10'	72° 36'	86,769	94,053	114,298	.810	1.111
0.88	123° 17'	82° 51'	70° 44'	84,841	91,963	111,758	.774	1.136
0.86	118° 38'	80° 34'	68° 54'	82,913	89,873	109,218	.740	1.163
0.84	114° 17'	78° 20'	67° 6'	80,984	87,783	106,678	.706	1.190
0.82	110° 10'	76° 8'	65° 18'	79,056	85,693	104,158	.672	1.220
0.80	106° 16'	73° 58'	63° 31'	77,128	83,603	101,598	.640	1.250
0.78	102° 31'	71° 49'	61° 45'	75,200	81,513	99,058	.608	1.282
0.76	98° 56'	69° 42'	60° 0'	73,272	79,423	96,518	.578	1.316
0.74	95° 28'	67° 37'	58° 16'	71,343	77,333	93,979	.548	1.351
0.72	92° 6'	65° 32'	56° 32'	69,415	75,242	91,439	.518	1.389
0.70	88° 51'	63° 31'	54° 50'	67,487	73,152	88,899	.490	1.429
0.68	85° 41'	61° 30'	53° 9'	65,559	71,062	86,359	.462	1.471
0.66	82° 36'	59° 30'	51° 28'	63,631	68,972	83,819	.436	1.515
0.64	79° 36'	57° 31'	49° 48'	61,702	66,882	81,279	.410	1.562
0.62	76° 38'	55° 34'	48° 9'	59,774	64,792	78,739	.384	1.613
0.60	73° 44'	53° 38'	46° 30'	57,846	62,702	76,199	.360	1.667
0.58	70° 54'	51° 42'	44° 51'	55,918	60,612	73,659	.336	1.724
0.56	68° 6'	49° 48'	43° 14'	53,990	58,522	71,119	.314	1.786
0.54	65° 22'	47° 54'	41° 37'	52,061	56,432	68,579	.292	1.852
0.52	62° 40'	46° 2'	40° 0'	50,133	54,342	66,039	.270	1.923
0.50	60° 0'	44° 10'	38° 24'	48,205	52,252	63,499	.250	2.000
0.45	53° 30'	39° 33'	34° 27'	43,385	47,026	57,149	.203	2.222
0.40	47° 9'	35° 0'	30° 31'	38,564	41,801	50,799	.160	2.500
0.35	40° 58'	30° 30'	26° 38'	33,744	36,576	44,449	.123	2.857
0.30	34° 56'	26° 4'	22° 46'	28,923	31,351	38,099	.090	3.333
0.25	28° 58'	21° 40'	18° 56'	24,103	26,126	31,749	.063	4.000
0.20	23° 4'	17° 18'	15° 7'	19,282	20,901	25,400	.040	5.000
0.15	17° 14'	12° 58'	11° 19'	14,462	15,676	19,050	.023	6.667
0.10	11° 29'	8° 38'	7° 34'	9,641	10,450	12,700	.010	10.000
0.05	5° 44'	4° 18'	3° 46'	4,821	5,252	6,350	.003	20.000

Expressed in another way we may say that the dry objective, where air is the medium between the objective and the cover-glass, catches a smaller per cent of light rays than the objective with water interposed in the place of air; or if we assume a maximum theoretical aperture of 180° for the dry objective, no

more light will pass through it from the object than through the water objective with an aperture of 97° , or through an oil immersion lens of 82° aperture.

The preceding table, copied from the pages of the *Journal of the Royal Microscopical Society*, is given to enable the student to compare his objectives with each other with but little trouble. This table was made out by means of the formula given above. "The numerical aperture of a lens determines all its essential qualities. The brightness of the image increases with a given magnification, other things being equal, as the square of the aperture; the revolving and defining powers are directly proportional to it; the focal depth varies inversely as the square and so forth." (ABBE.)

From what has been said in the previous pages we may classify objectives not only into *dry* and *immersion*, but, when we take into consideration the remarkable evolution which has taken place within recent years in the development of these important parts of the microscope in overcoming chromatism and spherical aberration, we may regroup them into two distinct systems called:—

1. Achromatic objectives.
2. Apochromatic objectives.

The achromatic objectives comprise the great majority of those used on better grades of microscopes until the discovery of the Jena glass. They give distinctness of image, but limited to one color of the light rays, the green-yellow; the other rays give confusion. The color correction is also limited to only one zone of the objective, and only two colors are united in that zone. It is therefore possible for the various colored images to fall on the same spot in pairs, but there is a considerable variation in the focus of each pair of colors. This result is accomplished by the combination of concave and convex lenses made of different kinds of glass (see page 6). The best of these objectives are also aplanatic.

The apochromatic objectives were devised by Abbe, of the

Zeiss Optical Company, and they give marked improvements over the older series or the achromatic. They are made to focus three colors in the same point, and color correction is uniform in all zones. "The amount of focal differences of the various sections of the spectrum, from the visible to far into the chemically active portion, are reduced to fractions varying from $\frac{1}{7}$ to $\frac{1}{10}$ of their original magnitude, *i.e.* they are practically eliminated, and this is done in equal degree for all zones of the objective. The images due to each single color, thus individually corrected, are rendered perfectly coincident and collectively form the final image."

With these objectives it makes no difference what may be the character of light which is emitted from the object; the quality of the image is not marred, but remains sharp and clear, and the natural colors of the object are faithfully reproduced, even to the most delicate tints.

There are other names given by opticians to objectives manufactured by them, but all the best objectives in the market will naturally fall under one or the other of the two classes given above.

Dr. H. van Heurck thus summarizes the chief forms of modern apochromats (*Ann. Soc. Belge de Micr.* XXIII, 1899, pp. 43-73. Also *Jour. Roy. Mic. Soc.*, p. 115, 1900):—

1. *Objective 16 mm., N.A. 0.30* (Fig. 24).—The system consists of three lenses: the frontal plano-convex of low curvature; the median double; and the superior slightly convex, and is in reality formed of a highly curved biconvex lens between two menisci. The low numerical aperture of this objective permits of employment only for histological studies, for which it gives very beautiful and delicate images.

2. *Objective 8 mm., N.A. 0.65* (Fig. 25).—This is a quad-

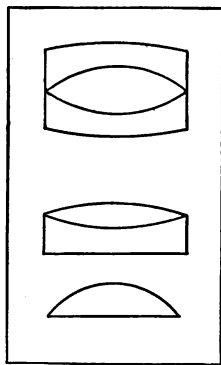


FIG. 24.—Objective 16 mm., N.A. 0.30.

rupture combination of seven simple lenses of different kinds of glass, and of different curves. It is a very successful objective, and if a microscopist possesses only one apochromat, he ought to select this. With different compensating oculars the magnification varies from 62 to 562 diameters. It shows Nobert's sixth group well with axial illumination, and resolves the striæ of *Pleurosigma angulatum*.

3. *Objective 6 mm., N.A. 0.95* (Fig. 26).—The construction of this objective resembles that of the last, but the curves

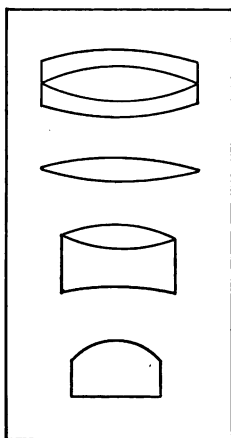


FIG. 25.—Objective 8 mm.,
N.A. 0.65.

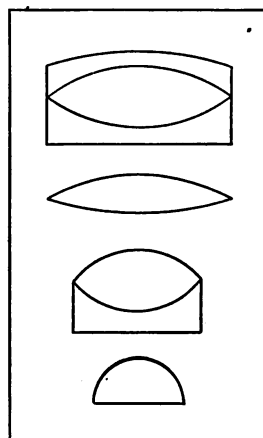


FIG. 26.—Objective 6 mm.,
N.A. 0.95.

are sharper, especially that of the frontal lens. It will revolve, with axial light, Nobert's twelfth group (2830 lines to the millimeter).

4. *Objective 4 mm., N.A. 0.95; and 3 mm., N.A. 0.95* (Fig. 27).—These resemble the last in design. The 3 mm. will resolve Nobert's thirteenth group, and it is somewhat superior to 4 mm.

5. *Objective 2.5 mm., N.A. 1.25, water-immersion* (Fig. 28).—Construction like last; a very beautiful objective.

A slight alteration of the diaphragm produces a marked effect on the image, and with axial light the resolution lies between Nobert's thirteenth and fourteenth groups. With electric oblique light Nobert's eighteenth group is distinctly resolved.

6. *Objective 3 mm., N.A. 1.40, homogeneous* (Fig. 29).— This contains ten lenses. The simple frontal lens slightly exceeds a hemisphere, and the object of such a lens is to produce a

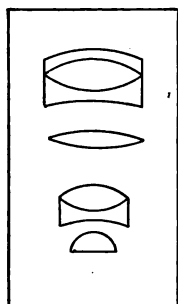


FIG. 27. — Objective 4 mm., N.A. 0.95.

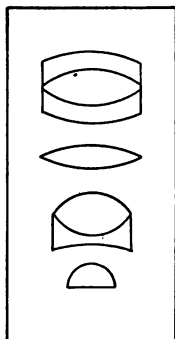


FIG. 28. — Objective 2.5 mm., N.A. 1.25.

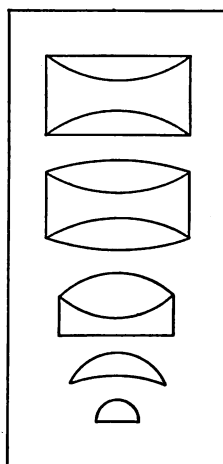


FIG. 29. — Objective 3 mm., N.A. 1.40.

notable amplification of the object without introducing at the same time chromatic and spherical aberrations. It reduces the pencil aperture from 1.40 to 0.65. The first doublet discharges similar functions; at first it diminishes the pencil divergence, and afterward intentionally introduces a certain amount of chromatic and spherical aberration for more complete correction of the upper lenses. The first triplet destroys spherical and chromatic aberration, and the second triplet the secondary spectrum.

LIST OF THE APOCHROMATIC OBJECTIVES

(ZEISS)

	Numerical Aperture	Equivalent Focus in mm.	Initial Magnification
Dry Series	0.30	24.0 ¹ 16.0	10.5 15.5
	0.65	12.0 ¹ 8.0	21 31
	0.95	6.0 ¹ 4.0 3.0	42 63 83
Water Immersion	1.25	2.5	100
Homogeneous Immersion	1.30	3.0 2.0 1.5	83 125 167
	1.40	3.0 2.0	83 125

TABLE OF MAGNIFICATIONS OF THE APOCHROMATIC OBJECTIVES WITH THE COMPENSATING EYEPieces

Calculated for an image distance of 250 mm. = 10 in.

(ZEISS)

Focus of the Objective	Searcher Eyepiece	Working Eyepieces					
	2	4	6	8	12	18	27
24.0	21	42	—	83	125	187	281
16.0	31	62	94	125	187	281	—
12.0	42	83	—	167	250	375	562
8.0	62	125	187	250	375	562	—
6.0	83	167	—	333	500	750	112
4.0	125	250	372	500	750	1125	—
3.0	167	333	498	667	1000	1500	—
2.5	200	400	600	800	1200	1800	—
2.0	250	500	750	1000	1500	2250	—
1.5	333	667	1000	1334	2000	3000	—

¹ The three objectives 24 mm., 12 mm., and 6 mm. of the dry series are constructed exclusively for the 10-inch tube, all the others are adjusted either for the Continental or English tube.

The practical value of the magnifications obtained with the high-power eyepieces in conjunction with lenses of relatively short focus is found by multiplying its number by the initial magnification of the objective. An objective of 3.0 mm. focus, for example, yields in itself a magnification of 83.3 (calculated for the conventional distance of vision of 250 mm.); eyepiece 12 in conjunction with this objective gives therefore a magnification of $12 \times 83.3 = 1000$ diameters. (ZEISS.)

It will be of some importance to the student to know, before making a purchase, how many objectives are required to enable him to perform his work with satisfaction and accuracy. Experience has demonstrated that the complete outfit for all investigations, from the ordinary to the most delicate work, must consist of the following objectives:—

- 2 inches (50 mm.).
- 1 inch (25 mm.).
- 1-2 inch (12.5 mm.).
- 1-4 inch (6.5 mm.).
- 1-10 inch (2.6 mm.).

In this series 4.2 and 2.0 mm. may replace the 6.5 and 2.6 mm. The 2.6 and 2.0 mm. objectives should be of the immersion type. The apochromatic lens, as has been stated, is the best, and it will be the part of wisdom to purchase this kind of objective.

For the use of students who are not far advanced in histology, the following objectives will be found sufficient:—

- 1 inch (25 mm.).
- 1-5 or 1-6 inch (5 mm. or 4.2 mm.).

How to use the microscope.—A delicate piece of apparatus in good condition is a most excellent assistant to the scientific worker; but if it is spoiled by careless handling, it will be a source of trouble ever afterward. The microscope when properly treated will be a faithful servant for many years, and will greatly aid the microscopist in solving many interesting problems which will be beyond his reach if the glasses and delicate parts have been previously injured by bad management.

Therefore if the student desires to become a successful investigator in Nature's laboratory, he must learn a few rules in relation to the use and care of the apparatus and diligently endeavor to apply them.

1. Never let the hands come in contact with any glass surfaces, particularly the lenses in the objectives and the oculars.

2. Keep everything about the instrument clean; eschew dust as one would the pestilence. If the lenses require cleaning, exercise the greatest care and gentleness in rubbing them, and use the softest old linen, or, better still, the Japanese paper, which is free of particles of grit. Do not permit alcohol to touch the metal portions of the microscope, because it will dissolve the lacquer with which the metal is covered. In order to locate particles of dust on the lenses, twist the tube of the microscope while the eye is looking through the ocular, and with the other hand hold the ocular stationary. If the particles revolve with the tube, it is a sure evidence that the dust is on the objective, and, vice versa, on the ocular.

3. If the tubes or other portions of the instruments need oiling, moisten a clean cloth with the best watchmaker's oil and rub the parts. Take care that none of the oil reaches the lenses.

4. While using chemical reagents it will be wise to protect all metallic apparatus from fumes of acids.

5. Never focus *downward*, for fear of injuring the objective lens or breaking the slide containing the object. Always focus *up*. In order to do this work correctly, bring the objective down by means of the rack and pinion movement, with the eye on a level with and looking across the stage; then it will be possible to lower the objective near the slide without bringing the two in contact. Now, with the eye looking through the ocular, focus up with the coarse adjustment until the image is brought into view and finish with the fine adjustment.

6. Begin work with the low powers of the microscope until an objective is found which will show the image of object distinctly in all its details. There is nothing to be gained in using a high power when a low power will do.

7. In using the immersion objectives, be certain to clean the lenses of all oil when the work is completed and before putting them away.

8. Chlorate of lime is an excellent absorbent of water, and when placed in the microscope case it will dry the atmosphere and thus prevent the rusting of the steel portions of the instrument.

9. The laquered parts of the instrument should be wiped dry and clean after the work is completed for the day, because the touch of the perspiring fingers will produce an indelible stain if left for several days.

10. If by accident the objective lens should come in contact with soft balsam or other resinous substances, remove the objective from the microscope at once and wipe off the gum with a soft cloth or with Japanese paper, moistened in benzol; dry at once with a fresh piece of paper before the solvent enters the mounting and dissolves the cement holding the lens in place.

Care of the eye. — There is so much danger that the student will seriously injure his eyes when he first begins to use the microscope, the teacher should take every precaution to prevent the trouble, and he should lay special stress upon the importance of properly adjusting the instrument and himself to the light. The field of the microscope should be well, but not brilliantly, illuminated; and direct sunlight must not be used for ordinary observation. The proper application of the diaphragm is important here.

The best light is secured from the sky or from a cloud. The observer will be in the best position for work when he sits facing the window, because then his hands will not be in the way of the illumination while he is manipulating the stage and its appliances. In this position the light may be painful to the eyes, at times, but this trouble can be obviated by placing a screen made of cardboard on the table in front of the observer. The lower edge of this screen should be raised above the table high enough to permit the light from the window to reach the reflector and stage of the microscope.

As soon as the eyes begin to pain or show indications of fatigue, the work with the microscope must be suspended and other duties should be assigned the student in order that the eyes may rest and recover their strength.

Use of the diaphragms. — These are disks, with different central openings, which are placed just below the stage and as near the object as possible. They are used for cutting off all rays of light reflected from the mirror except those which are illuminating the object. The correct use of the diaphragm must be determined by experiment, and its best effects are only secured by experience. As a general rule it may be stated that the diaphragm, when used without the condenser, must be well opened for low powers which have low apertures. When used with the condenser the object is to narrow down the pencil of light coming from the condenser, and in this case the diaphragm should be small for low powers and large for high powers.

Dark ground illumination is obtained by adapting a diaphragm with the opening in the shape of a ring and the centre oblique. This form of opening permits of oblique lighting and renders the field dark while the image stands out bright and striking. Dark ground illumination is also secured by making the diaphragm eccentric, that is, by moving it to one side.

On many of the microscopes now manufactured the iris diaphragm is placed, and this form enables the observer to increase or diminish the size of the opening at pleasure and with but little trouble. The term "stopped down" is generally used to designate the action of the diaphragm.

CHAPTER III

APPARATUS AND ACCESSORIES

BESIDES the microscope mentioned in preceding chapters, the laboratory should also contain the following accessories :—

The microtome.—There are several first-class instruments for cutting sections sold by some of the leading manufacturers, and, since they are all made on the same general principle, it will be only necessary to describe two or three which possess the most convenient facilities for work.

The microtome is an instrument constructed for cutting thin sections of animal and vegetable tissues, so that a satisfactory examination of the cells may be made with the microscope. The essential features of this instrument are a keen-edged knife firmly held on a sliding carrier, and a clamp for holding the specimen while the sections are being cut.

Before the microtome became the necessary adjunct to the laboratory, the method of cutting sections was by holding the specimen between the thumb and forefinger of the left hand and by a dexterous sweep of the razor held in the right hand. After considerable practice the student was able to secure sections of remarkable thinness.

The next step in the development was by Valentine's knife, which consisted of two blades parallel to each other and separated by screws. These screws permitted the adjustment of the blades, so that they could be separated at small distances. This knife was soon discarded by microscopists as inadequate to the demands.

The hand microtome, shown in Fig. 30, next came in favor, and is still used in some laboratories where a cheap form of instrument is required. This microtome is so constructed that

the specimen can be clamped in the well *a* by means of the screw *b*. The knife held in the hand slides over a glass plate, and the feed at *c* lifts the object through a minimum distance of ten microns. In the hands of skilful students this instrument will yield satisfactory results.

An improved form of the hand microtome is shown in Fig. 31, which is constructed to clamp on the edge of the work-table by means of the screw *a*, and the specimen is fastened in the instru-

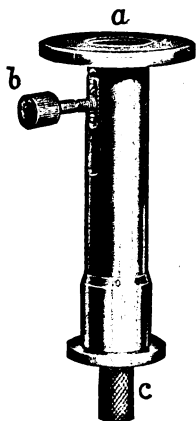


FIG. 30. — Hand Microtome.



FIG. 31. — Hand Microtome with Clamp for Table.

ment by the thumb-screw *b*. On top of the microtome are two glass ways along which the knife moves, and the milled head *c* raises the specimen to the plane of the knife. Sections can be cut with this microtome as thin as five microns. An ordinary razor can be used with this apparatus.

No instrument belonging to the microscopist's outfit has undergone such marked changes as the microtome. It has developed from the simple hand instrument to a microtome of great precision. There are many different patterns on the market, but the entire number may be classed under two heads, differing in the method by which the object is raised to the plane of the knife : —

1. Microtomes distinguished by having the specimen raised to the knife by means of a micrometer-screw in a vertical direction, or modifications of this movement. The Bausch & Lomb Optical Company's instruments are types of this class.

2. Microtomes distinguished by raising the specimen to the knife by means of an inclined plane. The Thomas's and Reichert instruments give illustrations of this class.

Bausch & Lomb's microtomes. — This firm makes several styles of microtomes belonging to the vertical micrometer-screw move-



FIG. 32. — B. & L. Automatic Microtome.

ment. The automatic microtome (Fig. 32) is their most elaborate instrument. "The object remains stationary during cutting, being held by an extremely rigid clamp adjustable in two planes and vertically. The knife is carried on a block, and is angular in section, sliding in perfectly ground ways so constructed as to give the greatest solidity with the least friction. The motion of the knife operates the feed-screw, leaving the hand free for the manipulation of the sections. The feed is from two to sixty

microns, in two microns. The carriage containing the feed arrangement and object carrier is adjustable along the whole front of the microtome, so that any desired cutting angle or length of stroke can be had. The split nut permits the object holder to be instantly lowered to beginning position when screw has been fed out. An automatic irrigator for keeping the knife flooded with alcohol or other fluid when cutting celloidin preparations consists of a glass cup on an arm adjustable over the knife. The flow of liquid is regulated by a screw-valve.

Minot's automatic rotary microtome.—This instrument, Fig. 33, was designed by Dr. Charles S. Minot, of Harvard University Medical School, and is well and favorably known in many labo-

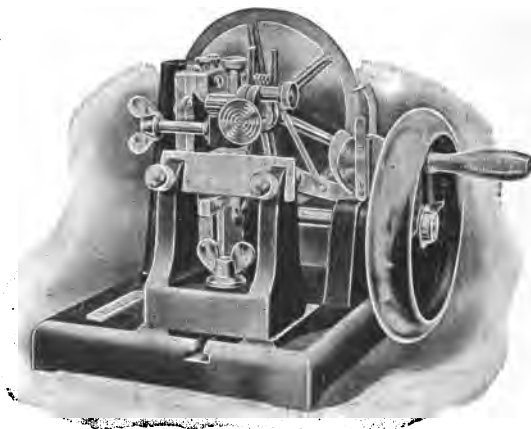


FIG. 33.—Minot's Rotary Microtome.

ratories. It is especially adapted for serial or ribbon sectioning. The object carrier is adjustable in three planes. The feed is so controlled that it will produce sections 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 microns in thickness. The object to be cut is cemented to the disk shown in the figure just above the knife. There is also an extra clamp for holding paraffin blocks which may be used on the machine

instead of the disk. The fly-wheel is accurately adjusted, so that it moves with the greatest ease and smoothness. A ribbon carrier, shown in Fig. 34, accompanies this microtome, and it is used for catching the serial sections. This rotary microtome is adapted only for cutting paraffin sections, and it works more rapidly than the precision instrument. The rapid movement of the wheel causes not only the disk carrying the object to approach



FIG. 34. — Ribbon Carrier.

the knife, but also an up and down rapid motion is given to the disk, so that the sections are automatically cut by the simple turning of the wheel. When the ribbon carrier is used in serial sectioning, the knife is transferred from its position shown in the microtome figure to the slots *a* and *b* on the ribbon carrier, and this latter apparatus is substituted for the ordinary knife holder shown in the figure illustrating the microtome. As the sections leave the blade of the knife, they move out on the ribbon in the order cut.

Minot's automatic precision microtome. — This instrument, Fig. 35, is totally different from the rotary microtome designed by Dr. Minot, and it is capable of cutting sections of uniform thickness of one micron. The object holder is the Naples universal clamp, which can be adjusted in two planes by rack and pinion. The vertical motion of the object carrier is accomplished by means of a fine adjustment screw and triangular prism similar to that used on the microscopes manufactured by the Bausch & Lomb Optical Company. This microtome is constructed for carrying the ribbon attachment used on the rotary machine.

upper end of the circular segment strikes against a metal cross-piece, which serves as a stop, and thus rotates the toothed wheel, and moves on the micrometer-screw so that uniformly thick sections are automatically obtained. When the micrometer-screw is run out, it can, in a circular notch of the vertical guide of the microtome, be rotated through 180° , and then commence again, the other end of the micrometer-screw being now in contact with the object slide. Thus the result is an uninterrupted working of this screw. The arrangement is especially useful in serial-section making." (*Jour. Roy. Mic. Soc.*, p. 59, October, 1901.)

How to care for the microtome. — 1. Keep the parts subject to friction well oiled, so that the carrier will move with the least difficulty. Use watch oil or pure paraffin oil of 25° .

2. Do not press on the knife while cutting, but permit it to move with its carriage by pushing and not pressing, because otherwise the bearing surfaces will soon become worn and uneven.

3. Keep the knife sharp as a razor, and see that no gaps occur on its edge.

How to sharpen the microtome knife. — For the accomplishment of first-class work the microtome knife, Figs. 37 and 38, must be in the best possible condition. It must be ground so

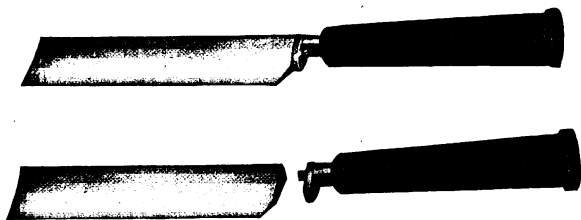


FIG. 37. — Microtome Knife with Handle for Sharpening.

keen that a hair resting on its edge will be severed. The same degree of care must be taken with this knife in sharpening it and in keeping it sharp that is taken with the razor in the hands of a first-class barber.

If the knife is in a dull condition, begin with the Belgian yellow hone, which is an open-grained stone, and it will cut the

metal to an edge quickly and will take out any gaps if such happen to be present. Lather the surface of this stone freely with palm-oil soap before applying the knife, and keep the surface well covered with the lather during the entire time of sharpening. This soap is preferable to oils ordinarily used for this purpose because it keeps the pores of the stone open and thus gives clean and free cutting surfaces. After the knife is properly ground on the yellow stone so that all gaps and inequalities are taken out of the edge, it is then ground on the blue-green water stone, which is a harder and finer-grained stone than the yellow

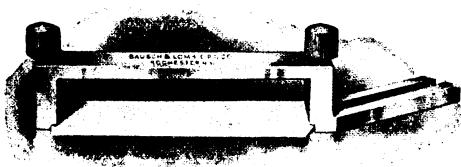


FIG. 38. — Knife mounted for the Microtome.

Belgian rock. On this stone the knife is sharpened until a fine thread is formed on the edge, which may be determined by passing the edge across the finger nail; when this thread is secured, the knife is passed over the stone without pressure until a uniform keenness is obtained along the entire edge, which may be determined by bringing the edge in contact with the moistened skin of the hand; and if the sensation is that the knife will enter the flesh, the grinding has been carried far enough. Of course, this grinding must be done in the same manner as that used in honing a razor, *i.e.* the edge must always be kept toward the front as the knife is passed back and forth on the stone.

When the proper edge is obtained on the knife with the blue stone, clean it thoroughly with a soft cloth, taking care not to bring the cloth in contact with the edge, and then strop it on fine leather covered with a prepared dressing. Pressure must not be great in this stropping, otherwise the delicate edge secured on the blue hone will be damaged. Draw the knife over the leather gently and lightly, and continue this work until a hair can be cut freely along the entire extent of the edge.

OLOGICAL LABORATORY METHODS

with a soft cloth, and the knife is then ready
ome. At frequent intervals during cutting the
he knife a few strokes on the strop, so that its
its keenness.

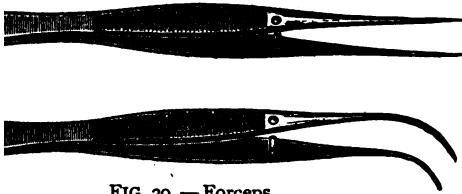


FIG. 39. — Forceps.

These are used for grasping the cover-glasses and
objects. The tips should be grooved on the
he objects may not slip out. There should be at
three instruments on each student's desk—one
s and the other bent (Fig. 39).

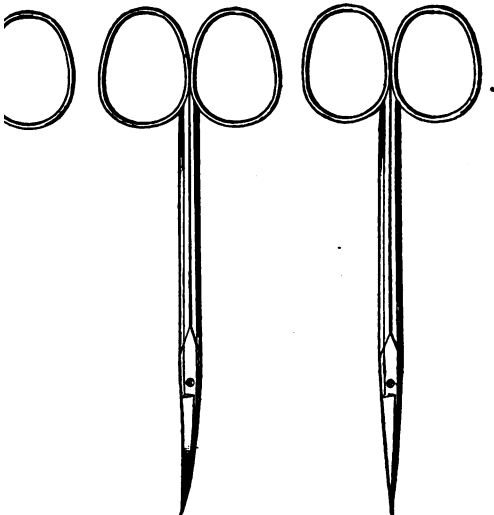


FIG. 40. — Scissors.

Scissors.

be either
venient (F
be kept keen



FIG.

Needle-holders. —
the sizes generally u
41) are so construct
tuted for another by
Needles may be purch
bent, and flattened poi
Scalpels. — Dissectiv
work, and various

facturers. The one illustrated in the cut (Fig. 42) is the best form for general use. It has an ebony handle and contains a blade 4.4 cm. long. The edge should be as keen as a razor at all times; and the surface of the knife must be polished after each using, so that rust may not spoil its working properties. The knife must be made of the finest English steel, well tempered, so that it will hold its edge under ordinary careful treatment.



FIG. 42.—
Scalpel.

Section lifters. — These are made of brass, nickel-plated, 1.9 cm. wide on the spatula end, and 13.1 cm. in length. This instrument is used for transferring the sections from one vessel to another without damaging their delicate structure (Fig. 43).

Spring-clips or compressors. — These are made of wire, nickel-plated, and are used for holding the cover-glass in position over the object while the slide is being cleaned and the mounting medium is hardening previous to spinning the final finishing ring.

Brushes. — Camel's-hair pencils in quills and mounted on short handles will be found useful in numerous manipulations: in transferring the delicate sections from the microtome knife to the watch-glasses; in moistening the surface of the knife while the sections are being cut; in dusting off cover-glasses and slips; in spinning rings of gum on the slips to seal the cover-glass. The pencils should be in assorted sizes, from Nos. 1 to 8.

Bell-glasses. — These are convenient for preserving the slides from dust during the process of mounting. These glasses should be from 10 to 30 cm. in diameter. A number of bell-glasses on the work-table will be found very useful.

Glass benches. — While the mounts are undergoing the various processes it becomes necessary to set them aside under certain conditions, and in a laboratory where many slides are being made, the space on the table must be economized. These

benches, therefore, when piled one on top of the other, and covered with a bell-glass (Fig. 44), will hold a greater number of slides than could be possibly stored under the bell-glass without them. The usual length is 130 mm., and 55 mm. wide.



FIG. 43.—Section Lifters.

When these benches are used the bell-glass should be procured of sufficient dimensions to accommodate them.

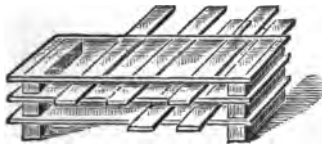


FIG. 44.—Glass Benches.

Petri dishes.— These comprise two glass vessels (Fig. 45), the larger inverted on the smaller, and they are used in inoculation experiments in the study of bacteria.

A small wash-bottle.— This apparatus, so necessary in a chemical laboratory, will be found useful on the microscopist's table for holding distilled water and dilute alcohol.



FIG. 45. — Petri Dishes.

Watch-glasses.— There are several shapes of watch-glasses sold in the market, but the author has used with satisfaction a pattern of watch-glass, called "Syracuse solid watch-glass" (Figs. 46 and 47), which possesses certain properties of special merit. The edges and bottom are accurately ground, so that when one glass is placed on top of another, air-tight covers are thus provided for each vessel. This form will,

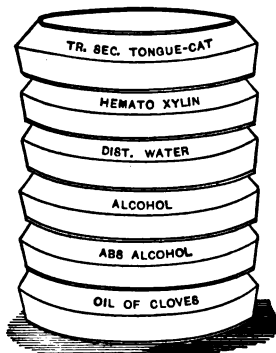


FIG. 46. — Syracuse Watch-glasses.

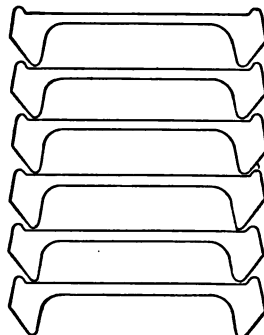


FIG. 47. — Section of Syracuse Watch-glasses.

therefore, take up less room on the table, which is quite a desideratum. The bevelled-ground edges form writing surfaces on which data concerning contents may be written with a pencil.

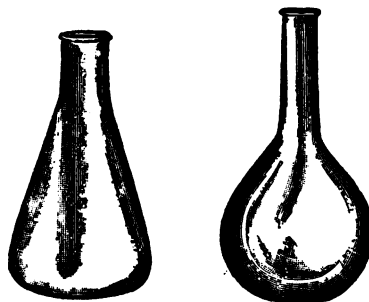
Glass pipettes, or dropping tubes.— These tubes (Fig. 48) are made with one end drawn to a point, and on the other end is a rubber bulb. They are useful for transferring a drop of fluid



FIG. 48. — Pipette, with Rubber Bulb.

to the places desired in the manipulations. By pressing the bulb and immersing the point in the liquid and then releasing the pressure, the fluid will rise in the tube and may be transported with perfect safety. By another slight pressure a drop or more may be exuded when desired.

Erlenmeyer's and Koch's flasks. — These vessels (Fig. 49), in various sizes, will be found serviceable in the laboratory. A supply of beakers will also be required for numerous purposes.



Erlenmeyer Flask.

Koch Flask.

FIG. 49.

Mortars. — Mortars made of iron, wedgwood, and glass should be placed in the laboratory for general use in the preparation of staining and mounting media.

Thomas's dehydrating apparatus. —

This, or a similar contrivance (Fig. 50), will be found useful in the preparation of the sections while staining, hardening, or dehydrating. The Thomas apparatus consists of a museum jar 20×21 cm. containing a diaphragm of perforated hard rubber suspended 76 mm. from the top. Through the holes,



FIG. 50. — Thomas's Dehydrating Apparatus.

of various sizes, in this diaphragm are passed the dehydrating tubes, which are 1.8, 2.5, and 3 cm. in diameter, and in which are placed the sections to be treated. On the lower ends of these tubes are fastened chamois skins. If dehydration is to be accomplished, the tubes are filled with 50 per cent alcohol, and in the jar is placed 95 per cent alcohol. By osmotic action the two will become the same strength, and the tissues can then be treated with absolute alcohol, if complete dehydration is desired. By this method of treatment the tissues do not shrink, since the changes are not sudden. An addition of calcium chloride to the jar's contents absorbs the water, and the quality of the alcohol is not depreciated. The rapidity of the dehydrating may be increased or diminished by the degree of thickness of the chamois skin on the tubes.

Turn-table. — This is one of the absolute essentials to each work-table. There are several patterns sold by dealers in

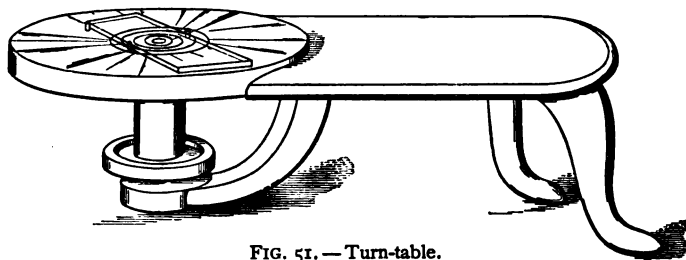


FIG. 51. — Turn-table.

microscopic apparatus, differing from each other in minor details, but the form illustrated in the cut (Fig. 51) contains all the important principles of the turn-table. This is the American table, and for all ordinary purposes it subserves the ends desired. Messrs. Bausch & Lomb have devised a table with a movable hand-rest, which projects partly over the circle of the table but not in contact with it, thus giving a support for the hand, which is very useful in those manipulations where steadiness of brush is necessary to produce neat and accurate work. Glass slips can be quickly centred on this table by means

of adjusting-pins, and the usual spring-clips are also present to hold the clip firmly to the table. The turn-table is used for spinning the cement rings on the slide to seal the mounts and exclude air from the specimen.

Water-baths. — There are so many things for which a water-bath is required in a well-equipped laboratory that it is deemed best to describe several kinds of apparatus adapted to this purpose.

The essential principle upon which these baths are built is a mass of water contained between an outer and an inner copper vessel. Heat applied to the outer vessel causes the water to boil and thus transmit a known degree of heat to smaller vessels placed on the bath or in its oven.

If the funds of the laboratory will permit of the expense, it will be best to provide each desk with one of these water-baths; but if the outlay of money is an important item and the director of

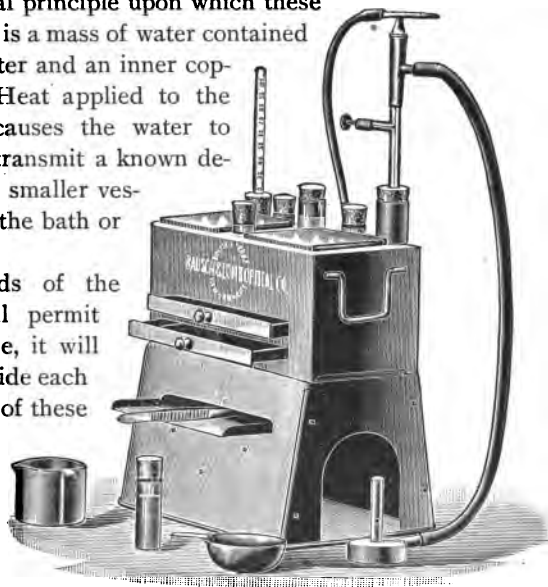


FIG. 52. — Miller's Water-bath.

the laboratory has not sufficient funds to furnish each student with a water-bath, then he should purchase one or more of the larger sizes which contain compartments enough for the class.

The bath devised by Dr. W. S. Miller of the University of Wisconsin (Fig. 52) is among the best of its kind for individual use. It consists of a rectangular, polished copper vessel with a sheet-iron base, to prevent the burning out of the copper. The top of the bath contains the following apparatus:—

1. Two cups, one 57 mm. in diameter and the same in depth, and the other 63.5 mm. in diameter, shallow, and shaped somewhat like a watch-glass.

2. Five bottles, to be used in various preparations of the imbedding material. As will be noticed in the cut, the bottles each occupy a receptacle sunk in the bath so that uniform heat may be secured.

3. Two drawers, or trays, in which the slides may be placed to dry and harden the gums.

4. A shelf upon which the forceps, needles, and other instruments used about the bath may be placed and kept at the same

temperature as that of the paraffin.

5. There is an opening at one end of the bath in which the Bunsen burner is placed.

6. A thermometer to keep the temperature uniform.

Another bath of value for individual use, devised by Dr. Reeves, is illustrated in Fig. 53. It consists of heavy polished copper, with

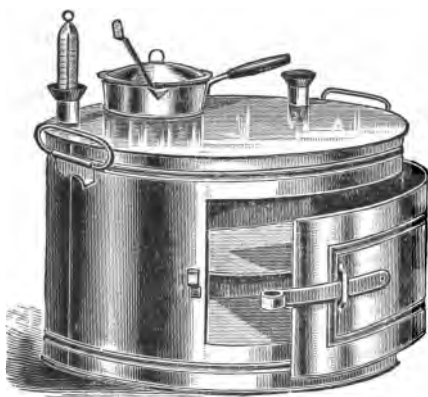


FIG. 53. — Reeves's Water-bath.

paraffin cup and stirrer and thermometer. The vessel is 216 mm. high and 254 mm. wide.

For general use in the laboratory a water-bath something like the Lillie pattern (Fig. 54) may be adopted with advantage. This apparatus was devised by Dr. F. R. Lillie, of the University of Chicago. The illustration gives a clear conception of this water-bath. It consists of two, four, and six rows of drawers in a large chamber. Each of the drawers is 25 cm. long, 10 cm. wide, and 8 cm. deep, and they are provided with partitions, and are numbered. The ends and bottoms are made of copper,

and the sides of perforated zinc. There are no partitions between these drawers, so that the heat may circulate through the perforated sides and throughout the entire large chamber.



FIG. 54. — Lillie's Water-bath.

In these partitioned drawers may be placed various vessels used in infiltrating, imbedding, or any other manipulation requiring heat for its proper completion. The large chamber consists of an inner and an outer vessel, between which the water is placed.

The outer vessel is made of a non-conducting material, so that an economy of heat is secured. A gas burner underneath furnishes the heat for

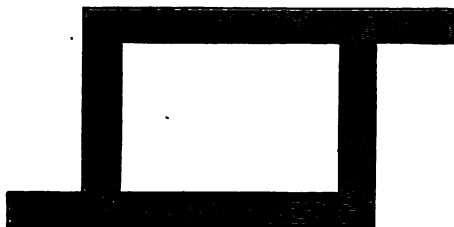


FIG. 55. — Paraffin Imbedding Box.

raising the temperature of the water, and a thermometer and a thermo-regulator are provided in tubulations, so that the degree of heat may be under control.

Paraffin imbedding box. — Where imbedding is required the apparatus shown in Fig. 55 is most convenient. It consists of two triangular pieces of type metal, about 20 mm. high, with a glass plate to form the base. To use the instrument, wet the glass plate with glycerin and gently warm it; now place the box on the plate, adjusted to the dimensions desired, and pour in the paraffin. Before the paraffin is cooled the specimen is immersed in it in the position desired; and after the preparation becomes hard, it may then be taken from the metal box and transferred to the microtome to be cut into sections.

Paper imbedding box. — This method is con-

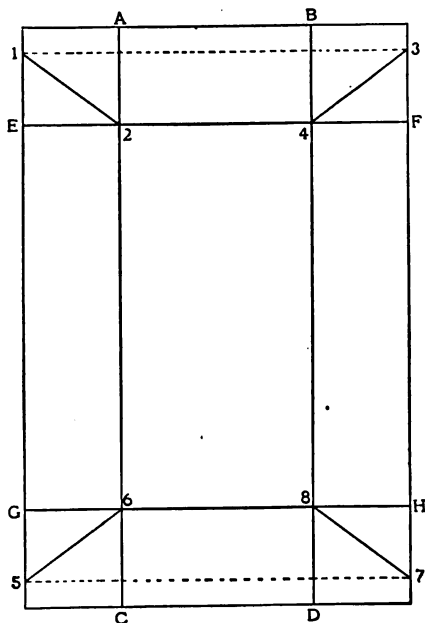


FIG. 56. — Paper Imbedding Box.

venient in some instances where the metal boxes are not available.

A piece of stiff paper, the size desired and in the proportions shown in Fig. 56, is bent in the *same directions* along the lines *AC, BD, EF, GH*; and in *opposite directions* along the lines 12, 34, 56, 78. Now fold the box in shape and turn over the ends along the lines 13 and 57, so that these laps will bind the box and thus prevent collapsing.

CHAPTER IV

PREPARATION OF THE TISSUE FOR MOUNTING

IN preparing tissue for permanent preservation, some definite order must be pursued or the results will not be satisfactory. The tissue must be cut into very thin sections, so that the cellular structure can be examined by means of transmitted light; but before these sections can be made, the specimen must be subjected to certain treatment preliminary to the cutting. In some instances the specimen, if plant or animal, must be killed quickly, so that the cells will not lose their normal condition; certain fluids and other compounds must be extracted from the specimen, in order that the chemicals used in the processes may not be interfered with in their action on the tissue; if the specimen is delicate and its tissue will be crushed in the jaws of the microtome, it will become necessary to strengthen the cell walls with paraffin or celloidin before the cutting can be successfully accomplished; in some cases the entire specimen must be stained before the cutting is done, and in those instances special treatment becomes necessary before the staining chemicals will penetrate.

The general steps for preparing the specimens for mounting and converting the fresh tissue into the completed slide for permanent preservation may be outlined as follows:—

1. When Paraffin is used:

Fixing and hardening the fresh tissue.
Dehydrating.
Clarifying.
Infiltrating.
Imbedding.
Cutting the sections.

2. When Celloidin is used:

Fixing and hardening the fresh tissue.
Dehydrating.
Treatment with ether-alcohol.
Infiltrating.
Imbedding.
Hardening with chloroform.

1. When Paraffin is used :	2. When Celloidin is used :
Fixing the sections to the slip and removing imbedding material.	Clarifying with castor-xylene.
Staining.	Cutting the sections.
Washing extra stain away with alcohol.	Fixing the sections to the slip and removing imbedding material.
Clarifying with carbol-turpentine, or carbol-xylene.	Staining.
Transfer to slip in Canada balsam.	Washing extra stain away with alcohol.
Place on cover-glass.	Clarifying with carbol-turpentine, or carbol-xylene.
Spin finishing ring.	Transfer to slip in Canada balsam.
Label.	Place on cover-glass.
	Spin finishing ring.
	Label.

Dr. Piersol in the Appendix to his Normal Histology gives the following method for preparing specimens for imbedding in paraffin, cutting, and mounting in Canada balsam : —

“ 1. Fixation of fresh tissue in large quantity by Müller's fluid; renewal when turbid; tissue remains 2-3 weeks.

“ 2. Thorough washing in running water 2-5 hours.”

“ 3. Transfer to 70 per cent alcohol; keep in dark; change alcohol whenever it becomes deeply tinged, until it remains colorless.

“ 4. Stain in excess of borax-carmines, 24-48 hours.

“ 5. Transfer directly, without washing, from stain to acid alcohol, 24-48 hours.

“ 6. Wash well in 70 per cent alcohol, several times renewed, 24 hours.

“ 7. Transfer to 80 per cent alcohol, 24 hours.

“ 8. Transfer to 95 per cent alcohol, 24-48 hours.

“ 9. Dehydrate in absolute alcohol, 24-48 hours.

“ 10. Transfer to pure chloroform until tissue sinks, 6-8 hours.

“ 11. Transfer to saturated solution of paraffin in chloroform, 6 hours.

“ 12. Transfer to pure melted paraffin, kept in constant temperature of about 50° C., until all chloroform is driven off 6-8 hours.

"13. Transfer to fresh melted pure paraffin of consistence for imbedding, 10-15 minutes.

"14. Imbed tissue in mould; cool rapidly.

"15. Section in microtome, first suitably trimming block for cutting.

"16. Fix sections to slides by gum or collodion clove oil.

"17. Remove paraffin by benzole, succeeded by turpentine.

"18. Drain off excess of turpentine, apply balsam, and cover.

"19. Place freshly mounted slide in horizontal position.

"20. Clean up and permanently label when thoroughly dry; store in suitable cabinet.

"While the duration of the several manipulations as indicated in the above summary represents the time usually required by ordinary objects, yet the individual character of the tissue must be considered in each case, as density exerts much influence on the rapidity with which the fluids penetrate.

"When it is desirable to stain the tissue after sections have been cut, the above manipulations must be modified; steps 4, 5, and 6 in such case are omitted, and the tissue is at once dehydrated. Removal of the paraffin from the fixed sections of the slides (17) by benzole, is at once succeeded by the following manipulations:—

"(a) Transfer to 95 per cent alcohol to remove benzol, 5-10 minutes.

"(b) Transfer to clean 95 per cent alcohol to insure complete absence of benzol, 5 minutes.

"(c) Transfer to 80 per cent alcohol, 5 minutes.

"(d) Transfer to 70 per cent alcohol, 5 minutes.

"(e) Stain in borax-carmin solution, 10-15 minutes.

"(f) Differentiate in acid alcohol (10 per cent), 6-10 minutes.

"(g) Wash in 70 per cent alcohol (renewed), 10-15 minutes.

"(h) Transfer to 80 per cent alcohol, 15 minutes.

"(i) Transfer to 95 per cent alcohol, 15 minutes.

"(j) Dehydrate thoroughly in absolute alcohol, 15 minutes.

"(k) Clear sections in oil of turpentine, 5 minutes.

“(I) Mount in balsam as indicated above in 18.

“When hæmatoxylin is used as the stain, the steps *e*, *f*, and *g* are omitted, and replaced by —

“(ee) Transfer to distilled water, 5 minutes.

“(ff) Stain in properly diluted hæmatoxylin fluid until sufficiently dark, 8–10 minutes.

“(gg) Wash well in distilled water to remove excess of stain and to differentiate, 10 minutes; then dehydrate by the ascending series of alcohols included by *h* to *j* as above.” (*Mic. Bul. and Science News*, April, 1894.)

Miller's scheme for imbedding, sectioning, staining, and mounting: —

1. When Paraffin is used:	2. When Celloidin is used:
80 per cent alcohol (<i>a</i>).	80 per cent alcohol.
Stain (<i>b</i>).	Absolute alcohol.
Wash.	Alcohol and ether.
Absolute alcohol.	Dilute celloidin.
Clear (<i>c</i>).	Saturated celloidin.
Paraffin.	Imbed.
Imbed.	80 per cent alcohol (<i>f</i>).
Section.	Section.
Fix on slide (<i>d</i>).	Stain (<i>g</i>).
Xylol (<i>e</i>).	Wash. (Then glycerin will terminate if used here.)
Absolute alcohol.	95 per cent alcohol.
Stain (<i>b</i>).	Eosin-alcohol (<i>g</i>).
Wash.	Oil of <i>origanum creticum</i> ; or oil of
Absolute alcohol.	cloves (<i>h</i>).
Xylol.	Balsam.
Balsam.	

“(a) If sections are to be stained on the slide after imbedding in paraffin, pass at once to absolute alcohol; if the specimen is to be stained *in toto*, pass to stain, etc.

“(b) For staining *in toto* use Grenacher's borax-carmine, alum-carmine, or Delafield's hæmatoxylin. For staining on the slide, use either the above, or, preferably, some one of the anilin colors. Specimens hardened in chromic or osmic acid mixtures take carmine stains badly.

"(c) Clear in either cedar oil, beech wood, creosote, or chloroform. Avoid clove oil, as it makes the paraffin granular.

"(d) If the specimen has been stained *in toto*, use Shalibaum's collodion clove-oil fixative; if sections are to be stained on the slide, use Mayer's albumen fixative.

"(e) If the specimen was stained *in toto*, pass at once to balsam; but if sections are to be stained, pass to absolute alcohol, stain, etc.

"(f) After imbedding and previous to placing in 80 per cent alcohol, it is well to place the block for a short time, 1-2 hours, in chloroform. This prevents the formation of bubbles and makes the celloidin more uniform in consistency.

"(g) For routine work, use Delafield's hæmatoxylin and eosin-alcohol; this gives a double stain. Any other stain may be used, but some anilins color the celloidin intensely. If other stain than the hæmatoxylin is used, the eosin-alcohol may be omitted.

"(h) If it is desired to remove the celloidin from section, clean the specimen in clove oil. With delicate section it should not be used." (*Amer. Mon. Mic. Jour.*, June, 1894.)

Staining in bulk (method after P. A. Fish in *Proceedings of American Microscopical Society*):—

1. Fixation of tissue.
2. Staining.
3. Dehydrating.

PARAFFIN METHOD

4. Cedar oil or chloroform.
5. Paraffin imbedding.
6. Cutting sections.
7. Fixing to slide.
8. Xylol.
9. Mounting in Canada balsam.

CELLOIDIN METHOD

- Ether-alcohol.
- Celloidin imbedding.
- Clearer (castor-thyme oil).
- Cutting sections.
- Mounting in Canada balsam.

Section staining:—

1. Fixation of tissue.
2. Dehydrating.

PARAFFIN METHOD

3. Cedar oil or chloroform.
4. Paraffin imbedding.

CELLOIDIN METHOD

- Ether-alcohol.
- Celloidin imbedding.

PARAFFIN METHOD

5. Cutting sections.
6. Fixing to slip.
7. Xylol.
8. Treatment with alcohol.
9. Treatment with water.

CELLOIDIN METHOD

- Castor-thyme oil.
- Cutting sections.
- Fixing to slip (ether-alcohol).
- Treatment with alcohol.
- Treatment with water.

10. Stain.
11. Water.
12. Wash in alcohol.
13. Clearer.
14. Mounting in Canada balsam.

All methods have the same general purpose and differ only in minor details, and these details are controlled entirely by the purpose in view and the character of the object to be cut.

Taking the scheme mentioned on previous pages as our guide, we will now proceed to give in detail the processes required to prepare and mount the section of the specimen for permanent preservation.

Killing, fixing, and hardening.—The three steps, killing, fixing, and hardening, may be accomplished in some cases by the use of one fluid, but in others it will require the use of two or three distinct solutions to reach the desired results. The killing should be done as quickly as possible to prevent the collapsing of the tissues and otherwise injury to the cells and their contents. The prime object of the fixing agent is to set the tissues in their natural forms as they appear during life; and the hardening agent is to give strength and tenacity to the cells for subsequent treatment.

Usually the same agent is adapted to both killing and fixing. It is best to have the material in small pieces, so that the fixing agent will penetrate thoroughly into all portions of the specimen. The volume of the agent should be about 15 to 25 times greater than the specimen to be treated. All fixing agents except alcohol must be washed out of the specimen before the succeeding steps are taken, because precipitates are often produced which greatly interfere with the proper examination of the tissues if this is not done. All aqueous agencies are washed out with

running water, while all alcohol fixing agents are washed with alcohol of the same strength as the agent. This washing out takes from twelve to twenty-four hours, but the time may be shortened by keeping the fluid lukewarm.

The following are some of the most important killing and fixing agents:—

KILLING FLUID FOR PLANT TISSUE (Schaffner's formula):—

Chromic acid	0.8 cc.
Glacial acetic acid	0.5 cc.
Water	99.0 cc.

Sixty cc. of this solution will be sufficient to kill one or two dozen objects of small size. The specimens should be immersed in the agent as soon as they are cut, and must be kept in it from twelve to twenty-four hours. This fluid is washed out by soaking in water from one to two hours in several changes.

Chromic acid and its combination with other chemicals give the most valuable killing and fixing agents for botanists. It is difficult to give exact formulæ for this agent, because opinions differ as to the effects produced on the plants, and it is best to try the experiment to determine the strength of the fluid needed. But the general formula given above may be relied upon for favorable results in most cases. This agent tends to harden the specimen, and some difficulty may be experienced in cutting the sections. Under such conditions it will be best to use another killing and fixing solution.

In the use of chromic acid it must be borne in mind that the agent combines chemically with the tissues, and the proper stain, therefore, to apply to the sections is hæmatoxylin or an anilin dye. Osmic acid produces the same results and must be followed with the anilin stains.

PERENYI'S FORMULA:—

Nitric acid	40 cc.
Chromic acid	30 cc.
Alcohol	30 cc.

Any alcoholic stain may follow this fixing agent.

FLEMMING'S CHROMIC ACID SOLUTION:—

Chromic acid of 1 per cent	25 vols.
Osmic acid of 1 per cent	10 vols.
Acetic acid of 1 per cent	10 vols.
Water	55 vols.

This formula is the stock solution. Lee in speaking of this fixing agent says, "I recommend that Flemming's mixture should be used wherever it is possible, as I believe it to be in general far the best fixing agent yet invented, with the exception of Hermann's mixture, which is unfortunately too expensive to be employed in large quantities." The above formula is the weak solution, but Flemming gives the following, which he terms his strong solution.

Chromic acid 1 per cent	15 parts
Osmic acid	4 parts
Glacial acetic acid	1 part

Lee states that on account of the large amount of organic acid given in these two formulæ they may not keep well, and he recommends that in the first formula the osmium be kept in 2 per cent solution in chromic acid of 1 per cent, and that 20 volumes of chromic acid, 5 volumes of osmium solution, and 65 volumes of water be taken to form the solution when required. In the last formula he recommends that it be made up from time to time from stock solutions, in which the osmium is kept separate from the acetic acid. In this case it will require two separate solutions as follows:—

A. Chromic acid 1 per cent	11 parts
Glacial acetic acid	1 part
Distilled water	4 parts

B. Two per cent solution of osmium in 1 per cent chromic acid solution; and when required, mix four parts of A with one of B.

HERMANN'S FORMULA, MODIFIED BY LEE:—

Platinic chloride 1 per cent	15 parts
Glacial acid 1 per cent	1 part
Osmic acid 2 per cent	4 or 2 parts

Protoplasmic structures are claimed by Hermann to be well preserved in this solution, better than in chromic mixtures. It does not cause precipitates as was stated with the chromic mixtures. Lee says, "On the whole, I take it that this mixture is *probably the most fixative yet discovered*." The objection to it is the great expense in its preparation.

MERKEL'S FORMULA (a very delicate fixative):—

Chromic acid and platinic chloride, equal volumes of 1:400 solution in water.

This agent was originally introduced for the study of karyokinesis (the transformations of the nucleus during cell-division), but it is excellent for general use in the case of delicate objects. It is readily washed out with 50 to 70 per cent alcohol. Stain with safranin or Kleinenberg's hæmatoxylin.

KLEINENBERG'S FORMULA:—

Picric acid (saturated solution in water)	. . .	100 parts
Sulphuric acid, concentrated	. . .	2 parts
Filter.		

Add three times bulk of water.

Add as much creosote as will mix.

This agent will kill quickly, remove sea-water entirely, and may be wholly replaced by alcohol. It has been much used at the Naples Aquarium. It is claimed by some to be superior to chromic acid solutions, because it does not produce precipitates, but leaves the tissues in good condition for staining. Large quantities of the fluid should be used, and it must be changed as often as it becomes turbid.

PICRIC ALCOHOL:—

Alcohol		250 parts
Picric acid		1 part
Water		250 parts

Fix for twenty-four hours, and then wash out with alcohol, soaking for a day in 70 per cent and then a day in 80 per cent alcohol. It is best to use saturated solutions of the picric

acid, because the dilute solutions are more apt to macerate the specimen. This fixing agent has great penetrating power, and yet it can be soaked out with alcohol, and tissues fixed in it may be perfectly stained in any dye.

PICRO-SULPHURIC ACID (Kleinenberg's and Mayer's formula) : —

Distilled water	100 vols.
Sulphuric acid	2 vols.
Picric acid, as much as will dissolve	(about 0.25 vols.).

The above is the concentrated solution. Kleinenberg's formula is obtained by diluting with three times its volume of water, and this is the form generally employed. Permit the specimen to remain in the fixing solution for three or more hours, and then place it in 70 per cent alcohol for five or six hours to remove the acid and harden the tissues; remove to 90 per cent alcohol and change again until the yellow stain due to the acid is entirely eliminated. Always wash out with alcohol. The specimens come out of this fixing agent with but little if any shrinkage.

RIPART'S AND PETIT'S CHLORIDE AND ACETATE OF COPPER FIXING SOLUTION : —

Copper chloride	0.39 grammes
Copper acetate	0.30 grammes
Acetic acid crystals	1 gramme
Camphor water	75 grammes
Distilled water	75 grammes

Lee considers this solution a most valuable agent for fixing cytological specimens, and it has the excellent quality of yielding fine results when followed with the stain methyl-green; the staining is almost instantaneous. The solution is specially recommended for those experiments where the tissues are fresh and hardening is not a required property. The osmium is added to the solution to strengthen the fixing action.

Formalin is one of the recent fixing agents, and is an excellent preservative. Wash out with water and follow by any stain.

Bichlorid of mercury. — This is a fixing agent of a high order, and, when properly applied, the tissues will yield to stains of any nature. In the case of carmine a brilliant color is produced because of the action of the mercury on the stain resulting in the formation of mercuric carminate. There are a number of combinations with corrosive sublimate, but Lee strongly endorses a saturated solution in five per-cent acetic acid, which he has found to be an excellent formula for marine animals. The most concentrated solution possible must be taken in every instance.

Do not place iron or steel instruments in contact with this agent, because precipitates will be formed which are injurious to the tissues. Wood, glass, or platinum can be used. The specimen must remain in this solution only long enough to fix, which may be determined by the degree of opaqueness the tissue assumes, thus showing the penetrating power of the corrosive sublimate. The time of fixing depends on the size of the object, extending from a few minutes to a much longer period. Wash out with either water or alcohol. Be careful to remove all trace of the sublimate, or otherwise the tissues will become brittle.

Alcohol. — There are two grades of this agent, absolute and alcohol diluted with two-thirds water. Use this fixing agent with tissues which are not apt to shrink easily. Absolute alcohol has a great affinity for water, and in the rapid dehydrating the delicate cells will collapse if delicate organisms are placed in it. A very large proportion of the alcohol must be employed for fixation.

Specimens to be examined for fatty matter should not be treated with alcohol, because this reagent dissolves from the tissues a portion of the fat, and therefore in the case of fatty degeneration it is best to examine the specimen in the fresh state. It is recommended by pathologists that it is best to use alcohol only in those cases where rapid diagnosis is demanded.

Hardening. — The methods of cutting in celloidin and in paraffin have now become so general among microscopists, soft tissues can be sectioned in the microtome, in most instances,

without first treating them in the hardening fluids, which was always resorted to before the imbedding methods became so universal. In a few instances hardening is required, and it is important, therefore, that the well-informed microscopist should know how to harden as well as how to imbed in paraffin or in celloidin.

Hardening should not be hastened, but the action should be slow, so as to prevent shrinking, and do not overharden. A large proportion of the agent must be employed, and the object must be suspended in it by means of a cord, so that any precipitates which may form will fall to the bottom of the containing vessel and adhere to the specimen. Frequent changing of the fluid will be beneficial. If a small portion of the hardening agent be employed, it will soon become saturated with the soluble matters in the object, and the hardening action will stop, followed by maceration of the tissues; hence the importance of using large portions of the fluid and in frequent changes.

Some of the best hardening agents are as follows:—

EHRLICH'S FLUID (after Müller):—

Bichromate of potash		2½ parts
Sulphate of copper		1 part
Water		100 parts

This solution will yield to the action of alcohol, and after the specimen has hardened it must be washed from four to ten days in alcohol. This solution is very good for soft tissue and where much blood is present. After transferring to the alcohol, place the vessel in the dark, because light develops crystals on the surface of the alcohol, and its penetrating properties are greatly reduced.

MERKEL'S FORMULA:—

Equal volumes of 1:400 solution of chromic acid and platinic chloride.

This formula is excellent for delicate objects, and it acts slowly, so that the specimens should remain in it for several days. Merkel allowed about four days for it to harden the

retina. Wash out with alcohol of about 70 per cent. Staining acts well after this hardening agent.

Alcohol. — Begin with a weak alcohol and follow with stronger until absolute strength is reached. This method will prevent too rapid action of the hardening agent, and shrinkage of the tissue will not result. Alcohol is not as good a hardening agent as either one of the above, but it will give valuable results when it follows the above formulæ. Pollen, starch, yeast cells, and spores after hardening in alcohol may be mounted in glycerin jelly with satisfactory results. After hardening place them in dilute glycerin and then mount in the jelly.

One of the disadvantages in the use of alcohol as a hardening agent is that it coagulates the albuminoids which produce a reduction in the transparency of the specimen.

In some specimens, like muscles and lung tissues, the alcohol does not harden, even after being immersed for a long period of time; but in these cases, if the specimens are placed first in dilute gum Arabic for twenty-four hours and then in the alcohol, the acacia is precipitated, and then the tissues harden. The gum can be removed by water.

Van Gieson¹ recommends the use of formalin as a hardening agent for brain and spinal cord. The solutions are made from 4 to 10 per cents, and the specimens are soaked ten days. Follow with alcohol in successive strengths.

Bacteria are best hardened in alcohol, because such agents, like Müller's fluid, produce dark granulations which are difficult to eliminate. Clear up by means of strong acetic acid.

Dehydrating. — The water found in all tissue is very deleterious, not only to the free penetration of the imbedding material required in cutting certain tissues, but its presence will cause the tissue to decay. Water also prevents the free penetration of balsam, which is the preserving medium and in which the specimen must be finally mounted. The usual method of procedure is as follows: —

The specimen is placed in rather diluted alcohol for a short time

¹ *Anal. Anscig.*, X, 1895.

and then transferred to a vessel containing a stronger quantity of the spirit, and so on until 95 per cent alcohol has been used. By pursuing this course of treatment there is slight danger of causing the specimen to shrink. Some manipulators use absolute alcohol for the last washing, but this is not deemed necessary in most cases, since the small amount of water remaining in the specimen after floating for a while in 95 per cent alcohol will be removed by the clearing agent.

In those cases where a fixing agent has been used, it is first important to remove this fixing agent before dehydrating is performed, unless alcohol is the removing agent, and then in that case it will partially dehydrate while it is removing the superfluous fixing substance. Care must be exercised in performing this part of the operation not to prolong the dehydration too far, because the alcohol will cause the tissues to become hard, brittle, and eventually shrivel up into an unnatural form.

Some authorities recommend the use of 95 per cent alcohol at once; and it is claimed that the weaker solutions of alcohol are not necessary, because if a large quantity of the strong alcohol is used, the water is washed out by the flooding of the dehydrating agent before the shrinking tendency is transmitted to the tissue. By using large quantities of 95 per cent alcohol the diffusion currents, which do so much damage to the tissues, are greatly lessened by the quick action of the surplus quantity of the dehydrating agent. Most microscopists, however, prefer to use the alcohol in different strengths as stated above, so as to avoid all chance of the violent osmosis which would cause a ruinous contraction of the delicate tissue. The specimen should remain in each degree of the alcohol from four to twenty-four hours.

Glycerin has been used by Overton for dehydrating, and he claims that the cells will not contract. This method consists in placing the specimen in 10 per cent glycerin, which solution is exposed to the action of the atmosphere when the gradual evaporation of the water will so concentrate the glycerin as to permit of the transfer of the specimen from it to strong alcohol without the danger of shrinkage.

Clarifying.— After the tissues have been thoroughly dehydrated, it is then necessary to remove the alcohol by replacing it with some agent which is readily miscible with the reagent used in the imbedding process, and this agent must also be anhydrous. This process is called *clearing* or *clarifying*. If the alcohol remains in the specimen, it will seriously interfere with the permeation of the imbedding substance and will seriously prevent the penetration of the balsam which is used to permanently preserve the tissue. Another object sought in clarifying is to render the specimen transparent.

It is very important, especially in the case of delicate objects, that the transition from the dehydrating agent to the clearing agent should be gradual, so that the tissues will not shrink but remain in their normal condition. Dr. Gierbrechts recommends the following method he has found to be efficient for accomplishing this gradual substitution of the clearing agent for the alcohol. A staining glass is partly filled with the clarifying fluid, and alcohol is placed on top of it. The difference in the specific gravity will cause the alcohol to remain on top for some time. The tissue is now placed in the vessel, and it will slowly sink through the alcohol into the clearing agent, and the former will slowly give place to the latter. The alcohol is now drawn off by means of a pipette. The specimen must remain in the clarifying agent until thoroughly clear; this will require from a few minutes to an hour, and, in a few instances, to several hours, the length of time being governed by the character of the object.

The following are some of the clearing agents used by the best microscopists:—

Anilin oil.
Bergamot oil.
Cedar oil.

Clove oil.
Origanum oil.
Turpentine oil.

Anilin oil is a strong agent, and it also acts quite well as a dehydrating agent. It will extract water from specimens which have been treated with 70 per cent alcohol. For certain kinds

of work it will be found exceedingly valuable — particularly for preparing specimens for paraffin imbedding. Unless this agent is removed by soaking for several hours in chloroform, however, the sections will turn yellow after a while.

Oil of bergamot does not destroy the anilin colors placed in the tissues, and it has the additional valuable property of clearing the specimens very quickly which have been dehydrated in 95 per cent alcohol.

Oil of cedar must be thin in consistency and of a light yellow color to give the best results. This oil evaporates slowly and mixes well with balsam dissolved in chloroform. It clears specimens very well and readily which have been dehydrated in 95 per cent alcohol. Mr. Lee says, in reference to this oil, "A laboratory equipped with cedar oil and origanum oil is fully equipped for all possible cases (the origanum oil being used merely to take the place of cedar wood oil for the special case of celloidin sections)." Cedar oil clears tissues stained in anilin dyes without extracting the color.

Oil of cloves is a very good clearing agent for certain kinds of work where minute dissection is resorted to. The specimen, however, cleared in it is very apt to become brittle after a short soaking in the oil, and this feature, in many cases, will be a decided disadvantage. The tendency to form minute drops over the slide gives it a value for minute dissection. Mixing it with oil of bergamot will reduce its tendency to produce brittleness in the tissues. It is an excellent clearer, and, when properly used, will be found to be a valuable addition to the laboratory equipment.

Oil of origanum. — This oil is valuable, since it will clear specimens which have been stained in anilin dyes without affecting the colors to any serious degree. It does not volatilize easily and rapidly, and is unchanged when exposed to the action of the light. The odor is not disagreeable. Celloidin sections are quickly cleared by it without dissolving the celloidin.

Oil of turpentine is only valuable for clearing specimens after they have been cut in paraffin. Its disadvantage consists in the

shrinkage it gives the cells, and in some instances, where alcohol is present, altering even the structure.

Infiltrating. — Paraffin with a melting point of 35° is melted over the water-bath and kept for some time heated only at its melting point. The specimen to be imbedded is placed in a solution of paraffin mixed with the clearing agent, and is then transferred into the hot paraffin, and here it is left until the paraffin penetrates all portions of the tissue. This is termed *infiltrating*. Generally it takes about one hour to properly infiltrate.

Of course if celloidin is to be used in the place of paraffin the infiltrating must be performed with the celloidin as the agent. The alcohol is removed in this case by ether-alcohol, equal volumes of sulphuric ether and 95 per cent alcohol, as the clarifying agent, and from the vessel containing the ether-alcohol the specimen is transferred to a thin celloidin solution ($1\frac{1}{2}$ grammes of collodion cotton dissolved in 100 cc. of ether-alcohol), where it must remain for two or three hours, depending upon the character of the specimen. It is then transferred to thick celloidin (6 grammes of collodion cotton dissolved in 100 cc. ether-alcohol). In this solution the specimen is left for three to five hours.

CHAPTER V

IMBEDDING METHODS

THE clearing process described in the preceding chapter is preliminary to the imbedding operation. This clearing work is necessary before the object is imbedded in paraffin, because otherwise the paraffin would refuse to enter thoroughly into all portions of the mass, so that proper cutting with the microtome would become difficult if not impossible. It is, therefore, important that the clearing work be correctly and thoroughly done, in order to secure satisfactory results with the imbedding material. The oil used in the process must penetrate all portions of the mass, and the water must be entirely eliminated, and the clearing medium must also mix well with the paraffin used in the imbedding work. With these simple facts well borne in mind, the student will proceed as follows to complete the imbedding of the specimen preparatory to cutting the sections.

Imbedding in paraffin.— This is the method for cutting small objects. Melted paraffin is substituted for the clearing substance by immersing the specimen in a vessel containing paraffin of the lowest melting point. The specimen is kept in the imbedding mass until it becomes thoroughly impregnated with it (infiltrating). The paraffin should be placed in one of the standard water-baths and kept just at the melting point. Duration of bath will depend on the nature of the specimen and the character of the clearing agent employed. Prolonged heating at any time is injurious to many forms of vegetation, and it should therefore be borne in mind that the employment of paraffin of low melting point and the reduction in the degree of heat, shortening as much as possible the time of the bath, will secure the best results.



After saturation has been accomplished, the specimen is transferred to the melted paraffin contained in the imbedding box described on page 64 and illustrated in Figs. 55 and 56, or in some similar box for the purpose, arranged as desired, and then cooled with water as soon as possible. Care should be exercised not to permit the water to enter into the warm paraffin, because if it does, the mass will at once become filled with holes and thus be injured for yielding good results in the microtome. As soon as cooled, the mass is shaped into a rectangular form, so that when placed in the microtome one face of the mass will be square with the knife and the opposite edge parallel with it. The microtome knife must not be moistened with liquid of any kind, but kept dry during the entire time of cutting the sections, so that rolling of the paraffin will be largely prevented. The temperature of the room should be made to sustain a certain relationship to that of the boiling point of the paraffin. In winter, when the melting point of the paraffin is 45° C., the temperature of the laboratory should be between 15° and 17° C.; in summer the relationship should be, for paraffin 48° , and 22° for the laboratory. The temperature of the paraffin, in winter, may be controlled by means of a reflector lamp, so placed as to have the heat rays reflected on the sections as they are being cut; in the summer this lamp may be replaced by a block of ice, with the reflector projecting the cool rays upon the mass of paraffin; or the room may be regulated in temperature by the adjustment of the window fixtures.

If it is desirable to produce in the cutting what is termed "ribbon-sections," or in series, it will be best to have in the laboratory one of the microtomes made for that purpose, such as Minot's automatic microtome, or any other of the same character; but it is possible to make these ribbons with the judicious use of the knife on any of the ordinary standard microtomes. The following hints are given to aid the student in accomplishing this end:—

1. The edge of the section is held down by means of a camel's-hair pencil until contact is made with the next section and the heat from the lamp causes it to cohere.

2. The block of paraffin must be pared down very close to the object and so cut as to present a square edge to the knife. The cutting must be rapid, so that the blows of the knife will slightly heat the paraffin, and thus cause the edges of the sections to cohere and form the ribbon.

3. The use of what is known as the "section stretcher," which is so placed as to rest on the upper surface of the knife, and holds the sections in a flat position and in contact with each other in the best way to form the chain.

In the case of those specimens which are so brittle or the parts of which are so slightly cohering that separation takes place during the cutting, the following method of procedure is recommended :—

A bottle of collodion is placed convenient to the microtome, so that a brush may be dipped in it and the surface of the paraffin covered with a thin coating of the collodion as each section is cut. The collodion must be of such consistency as to dry quickly without leaving a polished residue on the section. This coating must be made very thin, or the results will not be satisfactory. Repeat the operation after cutting each section, and the collodion will be found to sink into the specimen and upon drying will hold the parts together. When the section is taken from the knife, it should be transferred to the slide with the collodion surface downward, in contact with the slide, which has been previously prepared with Schaillibaum's fixative. ("One part of collodion is shaken up with three to four volumes, according to the consistency of the collodion, of clove oil or lavender oil. This should give a clear solution. A little is spread on a slide with a small brush. After arranging the sections on the prepared surface, warm over a water-bath, gently, until the clove oil has evaporated—five to ten minutes."¹)

For cutting ribbons Adam Sedgwick proposes the use of softer paraffin around the block of harder paraffin in which the specimen is imbedded. After the imbedding has been accomplished, the block is dipped in softer melted paraffin and the surplus

¹ Lee's "Microtome's Vade-Mecum," p. 212.

is cut off, leaving only a thin layer of the coating on the front and rear faces of the block. When the cutting begins, this soft paraffin will cause the sections to unite into the ribbon form.

In the laboratory there should be found two grades of paraffin: one melting at a temperature of 45° to 46° C. and a hard paraffin melting at a temperature of 55° to 56° C. The microscopist can then mix these two together in proportions to suit the condition of temperature in the room. It must be remembered that the harder the paraffin the thinner may the sections be cut, but there is stronger liability to roll. It is best, therefore, to mix the two kinds in such proportions as to produce a paraffin which will give a melting point of 50° to 55° C. This will have to be determined by experiment, but ordinarily the combination may be secured by mixing in the proportion of 20:9.

Plant tissues vary so much it is necessary to use great care in their treatment to prevent shrinkage while passing through the several liquids preparatory to imbedding for paraffin cutting. In the case of those specimens which contain much meristemic and watery thin-walled tissue, the following procedure is recommended by Dr. J. W. Moll in the *Botanical Gazette* for January, 1888.

The dehydrating of the specimen was accomplished by using a solution of chromic acid and 20 per cent, 35 per cent, 50 per cent, 75 per cent, and 90 per cent alcohols. The chromic acid acted on the protoplasm so as to fix it and macerate the cellulose, and thus permit the alcohol to permeate more freely. The dehydration must be done by slow degrees, so it is best to permit the specimen to remain in the several alcohols for twelve to twenty-four hours, depending upon the size of the specimens under treatment. Passing from the alcohols, the plant is placed in a solution of equal parts of turpentine and paraffin. This solution is now raised from a temperature of 20° C. to 45° C. The specimens are then placed in melted paraffin, which is retained at a temperature of 50° . It will take from one to two hours to permeate the specimens.

Mr. Gustav Mann, in the "Transactions and Proceedings of

the Botanical Society of Edinburgh,"¹ thus describes a method of preparing vegetable tissue for paraffin imbedding:—

"Requisites: (1) Picro-corrosive alcohol. Heat absolute alcohol to 50° C., saturate with picric acid, and then add bichlorid of mercury to saturation. When cool decant. This solution may be made in quantity and kept. (2) Absolute alcohol. (3) Chloroform-alcohol, chloroform, and absolute alcohol mixed in equal parts. (4) Chloroform. (5) Solid paraffin, melting point 46°–50° C. (6) Short, wide-mouth bottles. (7) Best cork stoppers, two for each bottle; one fitted with a piece of glass tubing 1 cm. in diameter and 3 cm. long. (8) Number of glass rods drawn out into fine points, as one must avoid bringing metal instruments in contact with the picro-corrosive fluid.

"Method: A. The fixing and hardening of tissues: Place the tissue in at least fifty times its bulk of the picro-corrosive alcohol. Leave small objects (up to 1 cubic cm.) for twenty-four hours, larger objects for forty-eight hours and upward, in the fluid. Keep the bottle well corked.

"B. The replacement of the picro-corrosive alcohol by pure absolute alcohol. (1) Pour off the hardening fluid till the tissues are just covered. Add absolute alcohol, according to the size of the tissue, in 1–10 drops every ten minutes till the tissue is again in fifty times its bulk of fluid. After each addition move the bottle very gently to allow the added alcohol to mix with the hardening fluid. Leave the tissue in this diluted mixture for twenty-four hours. In no case should this process be hurried, or strong diffusion currents will be set up, and the protoplasmic contents of the cell separate from the cell wall. (2) Pour off the fluid till the tissue is just covered, and add absolute alcohol to the original bulk. Move about the bottle every three or four hours. Most of the picro-corrosive material will thus be extracted after twenty-four hours. (3) Draw the fluid off rapidly by means of a pipette, and add absolute alcohol up to half the original bulk. Any drying of the tissue must be carefully guarded against. Leave for twenty-four hours and repeat the process.

¹ *Jour. Roy. Mic. Soc.*, p. 686, 1891.

"C. The replacement of the alcohol by chloroform: (1) Pass, by means of a pipette, the chloroform-alcohol mixture to the bottom of the vessel, when the tissue will float on the mixture; remove then the superfluous alcohol by a pipette, leaving only enough to cover the tissue. (2) When the tissue has sunk in the chloroform-alcohol mixture, introduce by a pipette pure chloroform, on which the tissue will float; the fluid above the tissue is removed by a pipette. After twenty-four hours the tissue may or may not have sunk in the chloroform; if not, it may be induced to do so by heating the chloroform to 20° C. (not higher); if this fail, a little sulphuric ether may be added. After the tissue has sunk, leave for twenty-four hours. (3) Place a fresh supply of chloroform at the bottom of the vessel (fifty times the bulk of the tissue), and if there is a distinct line of demarcation between the newly added and the old chloroform, the upper layer should be removed by a pipette.

"D. The replacement of the chloroform by paraffin: (1) Place the tissue in a warm chamber, heated to 25° C.; add solid paraffin in pieces up to the size of a small pea. After each piece has dissolved, the bottle has to be moved about very gently to hasten the mixing of the paraffin, which will be in the upper layers, with the chloroform. Continue until no more paraffin dissolves. Tissue which did not sink in pure chloroform will always sink as soon as paraffin is added. (2) Place the tissue in a warm chamber heated to 30° C. for twenty-four hours. (3) Place the tissue in a warm chamber heated to the melting point of the paraffin (46° C.), and after six hours replace the ordinary cork stopper (which up to this stage has always to be employed) by a perforated one. This method is adapted to insure a gradual giving off of the chloroform, for I find that, if the latter be driven off rapidly, a good deal of shrinkage always results. When all the chloroform has evaporated, — *i.e.* if after shaking the bottle gently one is unable to detect, by smelling, the faintest trace of chloroform, — then the tissue is ready for sectioning. (4) The tissues should not be exposed longer than just necessary to the temperature of melted

paraffin, but should be imbedded by means of Leuckart's type-metal box."

Herr F. Rosen gives the following method for imbedding vegetable tissue so as to produce the least contraction possible:¹—"The objects, thoroughly dehydrated in absolute alcohol, are successively transferred (1) to a mixture of equal parts of absolute alcohol and bergamot oil; (2) to pure bergamot oil; (3) to a mixture of equal parts of bergamot oil and paraffin; (4) to paraffin with melting point of 45° C.; (5) to paraffin with melting point of 56°-58° C. for twenty-four hours.

"In stages (3) and (4) the fluids should be kept at 48°; in stage (5), at 60°. In order to make the sections adhere to the slide, they are placed, while still in paraffin, on a drop of fluid which can be evaporated completely, such as distilled water, or 50 per cent pure alcohol. The paraffin is dissolved out by xylol. The alcohol must be completely evaporated before the treatment with xylol, otherwise the sections will get free."

Imbedding in celloidin or collodion.—This method is adopted for cutting large objects, while paraffin is used for small objects. This is necessary, because if paraffin was substituted for celloidin in the case of large objects, of ten millimeters or more, perfect sections would not be secured, since the paraffin has a tendency to split when the knife strikes it. On the other hand, celloidin will not yield as thin sections with small specimens as those produced by paraffin and is not suitable therefore for minute anatomy.

Celloidin is a preparation of pure pyroxylin (nitrocellulose), manufactured in Germany and sent out in the form of tablets of a tough, gelatinous consistency. It is soluble in ether and absolute alcohol. Imbedding in collodion was first introduced by Duval, in 1879, and it was afterward improved on by the introduction of celloidin. The method recommended in the use of celloidin is as follows:—

The object is first thoroughly dehydrated in absolute alcohol, and then soaked in ether until saturated, or in a mixture of ether

¹ *Jour. Roy. Mic. Soc.*, p. 632, October, 1894.

and absolute alcohol. This will usually require several days. From this solution it is transferred to solutions of celloidin, of various degrees of consistency, beginning with a thin solution and passing from thinner to thicker until the specimen has been thoroughly impregnated with the celloidin. Busse proposes using three solutions: the first consisting of 10 parts, by weight, of celloidin and 150 parts of ether and alcohol mixture; the second, consisting of 10 parts of celloidin and 105 parts of the ether-alcohol mixture; and the third consisting of 10 parts of celloidin and 80 parts of ether-alcohol mixture. The object must remain in the first bath for a length of time sufficient to permit of thorough penetration. To accomplish this may require several days. After soaking in the last solution, the specimen is imbedded in the celloidin by either one of the following methods:

1. The imbedding material is moulded in the paper box, or metal box, mentioned on page 64. The bottom of the box is covered with a layer of the thick celloidin and allowed to dry by evaporation; another layer is placed on this and allowed to dry; and so on until a sufficiently thick coating is secured in the bottom of the box. The object is now arranged on this coating of celloidin and fresh solution added until the object is entirely covered with the imbedding mass to the depth desired. After the filling has been completed, the box is placed under a bell-glass resting on a ground-glass plate and the celloidin allowed to slowly dry in the alcohol atmosphere generated from the block of imbedding material. When sufficiently hard to resist the pressure from the ball of the finger, the paper or metal box is removed and the mass is immersed in chloroform, where, in a short time, it assumes the form of a hard coagulated body resembling wax and beautifully transparent. After coming from the chloroform it is placed in a vessel containing white oil of thyme, or similar oil, where it will assume, in a few hours, the appearance of a clear glasslike mass.¹ It is best to use the chloroform immersion for small objects, but for larger bodies it is recommended by many microscopists to use alcohol of 85°. In

¹ *American Naturalist*, XXVI, p. 80, 1892.

the 85° alcohol solution the block of imbedding material must remain until it assumes the consistency required for good work in the microtome. This may take several days. The vessel containing the alcohol must not be closely corked. After the mass of celloidin has been well hardened, it can be preserved for a long time in 70° alcohol.

While the block of celloidin is being cut, it must be kept moist with 70° alcohol, since it dries very rapidly. If, however, the oil of thyme treatment has been resorted to, the knife of the microtome may be lubricated with this oil, thus making it possible to cut thinner sections. Moreover, the oil does not evaporate quickly like the alcohol, and the operation may be stopped for some time without serious detriment to the celloidin mass.

If the object has not been stained in mass, it may be difficult to distinguish it in the mass of transparent celloidin. Under this condition it will be best to slightly color the celloidin with picric acid or other similar stains.

When submitted to the pressure of the microtome clamps the celloidin yields slightly, and there is danger of distorting the object and thus destroying its histological value. To avoid this trouble, the block of celloidin, after being properly hardened, is mounted on a piece of wood suitably shaped for being grasped by the microtome. A coat of the thick solution of celloidin is spread over the surface of the wooden block and allowed to dry. The under surface of the celloidin block is trimmed to expose fresh surface and a solution of the celloidin smeared over it. The two surfaces thus prepared are pressed together, and then placed in a solution of 70° alcohol to soak for a few hours, in order that the point of contact may become hardened. The wooden block is now clamped in the microtome and the sections cut in the usual way.

2. This method consists in building up the block of celloidin on the wooden support from the start. The surface of the wood is slightly roughened, so that the material will stick securely, and a layer of the thick celloidin is spread over it and permitted to dry, and then another layer is added, and the process repeated

until the foundation is built up high enough, when the object is put into position and the celloidin placed around and over it until it is entirely covered with the imbedding mass. The hardening of the celloidin and after treatment are the same as used in the first method.

Herr O. Jelinek¹ recommends the use of the new insulating material called stabilite in the place of the wooden support for celloidin imbedding. Its advantage consists in its insolubility in water or alcohol (wood and cork are slightly soluble in alcohol, giving up coloring matters and tannic acid); it can be cut by a saw into any shape desired; it is firm and will withstand the pressure of the microtome; it is cheap; it may be readily written on with pencil or ink, which are not easily effaced while under treatment; it is not hygroscopic and will quickly sink in alcohol, thus bringing the imbedding mass in thorough contact with the hardening solution; it is not attacked by dilute hydrochloric and sulphuric acids.

It is important, says Dr. A. Elsching,² that celloidin solutions should be free from air and water. To effectually deprive celloidin of water, the tablets should be cut up into little blocks, the sides of which are not bigger than 5 mm. These are placed between folds of blotting-paper, and first allowed to dry at the room temperature, and then desiccated in an incubator. At this stage they should be of a yellowish hue and of horny consistence. Absolute alcohol may be easily obtained entirely free of water, by repeated treating with freshly dried copper sulphate. The dried cubes are placed in a narrow-necked bottle, with air-tight stopper, and form a layer not exceeding one-fourth the volume of the bottle. The celloidin is then just covered with the absolute alcohol, and allowed to stand for about twenty-four hours, after which the ether is poured in. In a very short time the celloidin is all dissolved, and thus, as no stirring is required, the solution is kept free from air bubbles.

¹ *Zeitschr. f. wiss. Mikr.*, XI, p. 237, 1894.

² *Jour. Roy. Mic. Soc.*, p. 404, June, 1894. And *Zeitschr. f. wiss. Mikr.*, pp. 445-446, 1893.

Freezing.—Freezing in the place of imbedding is often resorted to by microscopists in cutting sections from soft tissues, and excellent results are generally secured. The specimen is fastened to the plate of the microtome by a gum, which is also frozen and firmly holds the specimen during the cutting of sections. In using this method, care must be exercised to prevent, as far as possible, the formation of crystals in the cells of the delicate tissues, so that rupture will not take place. This trouble is greatly overcome by the use of some gummy substance which will not form crystals when the specimen is frozen. The gum is infiltrated into the specimen. It is recommended in the *Journal of the Royal Microscopical Society* that, when the specimen is soaked in oil of aniseed for some hours—twelve to twenty-four—the freezing may be safely accomplished without detriment to the tissues. The oil is dissolved out of the sections by the use of alcohol. The freezing is successfully done by the apparatus illustrated and described on pages 94 to 98.

Freezing microtomes for use with carbon-dioxid tanks.—(Condensed from an article written by Professor C. R. Bardeen, of Johns Hopkins University, for the *Journal of Applied Microscopy*, Vol. IV, p. 1320.)

Of late carbon dioxide has been much utilized, especially by pathologists, as a means of freezing tissues for sectioning. The convenience with which fluid carbon-dioxide may be obtained in tanks, and its power of rapid freezing, have caused it to be preferred to ether and similar fluids. In every active pathological laboratory the freezing microtome is in daily use. Perhaps its greatest value lies in the fact that thin sections may be made within a few minutes after the removal of the tissue from the body, and in a few minutes more these sections may be hardened, stained, cleared, and mounted. The surgeon may thus be given a positive diagnosis of the microscopic condition of the diseased tissues while he proceeds with the operation.

The carbon-dioxide microtomes commonly used have, however, several drawbacks, which have served to render them far less useful than they should be. From the practical standpoint their

most serious drawbacks are a tendency to become clogged and a great wastefulness of gas. From a scientific standpoint lack of control over the temperature of the freezing stage serves to give rise to an "over-freezing," which produces marked alterations in the tissues. In order to remedy these defects the machine described below was devised.

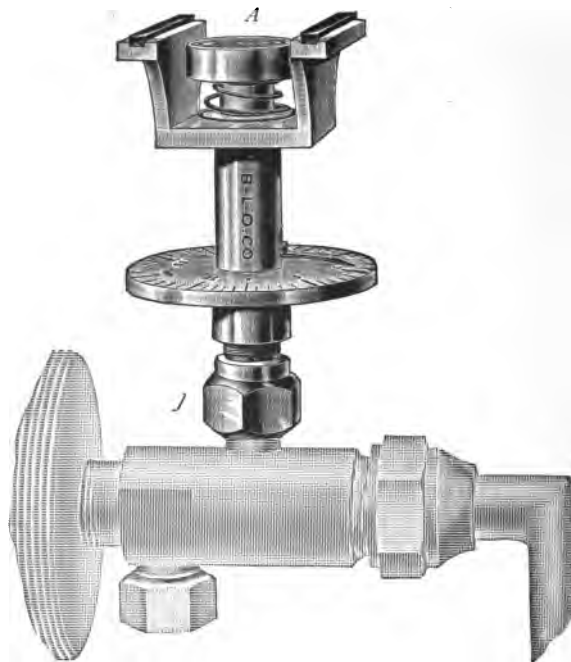


FIG. 57. — Bardeen's Freezing Microtome.

Figure 57 shows the machine as it stands ready for use. It is supported directly by the nozzle of the carbon-dioxid tank. This offers a firm and convenient means of attachment, but, if desired, a heavy tubing may be utilized to connect tank and machine. When the microtome is screwed directly upon the carbon-dioxid tank, it is necessary that the latter lie in a horizontal position. On the other hand, if an L-shaped piece of

tubing be utilized to connect freezing microtome and tank, the tank may be placed at any desired angle.

The valve of the tank is used to control the escape of gas into the machine.

The axis and main support of the instrument consists of a stout tube with a narrow lumen (*K-D*, Fig. 58). This axial tube is united by a nut (*J*, Figs. 57 and 58), either directly to the nozzle of the tank, or, in case a connecting tube is used, to the latter.

On the top of the axial tube the freezing stage (*A*, Fig. 57; *A-C*, Fig. 58) is screwed. This stage piece consists of two parts, a base and a cover. The base is the part screwed into the upper end of the axial tube (*C*, Fig. 58). To this base the cover piece is screwed (*A*, Fig. 58). Between the base of the stage and the axial tube is placed a thin brass plate (*D*, Fig. 58), with a very narrow aperture at its centre. Through this narrow aperture the carbon dioxid escapes into the lumen of the stage piece (*C*, Fig. 58). The difference in pressure on the two sides of the brass plate causes a very rapid expansion of gas between the cover and base of the freezing stage. The passage open for the escape of gas from the lumen of the base (*C*, Fig. 58) to the external world is in the form of a spiral passage, which finally opens out through the side of the cover, as shown in Fig. 57, *A*. Between the cover and base of the

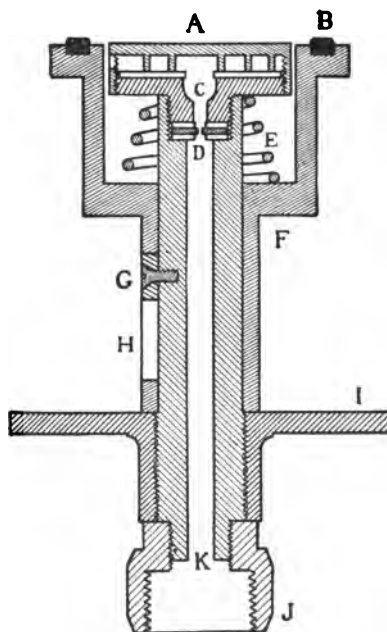


FIG. 58.—Section of Bardeen's Freezing Microtome.

freezing stage an asbestos washer is placed. The expanding gas, therefore, can absorb little heat from the base of the stage. Almost all heat absorption must take place from the cover. This heat absorption is greatly facilitated by the metallic spiral, which projects down from the cover so as to give rise to the spiral passage through which the gas escapes.

Through the mechanism here described, far the greater part of the heat-absorbing power of the expanding gas is utilized to lower the temperature of the surface of the cover of the freezing stage. The temperature of the rest of the machine is but little altered. Good control of the temperature of the freezing stage can be thus maintained. This control is further rendered possible by the valve of the tank. If this valve be turned on full, the temperature of the cover of the freezing stage will be quickly reduced to a very low point. Tissue placed on it is quickly frozen. On the other hand, if the gas is not permitted to escape from the tank with full force, the difference in pressure on the two sides of the brass plate is less and heat absorption from the cover is less marked. In this way tissues placed on the cover may be slowly frozen without subjecting them to severe cold. Thus, too, a constant low temperature may be maintained by opening the tank valve to the required point.

The mechanism for controlling the thickness of the sections is equally simple. On the lower end of the axial tube a movable wheel (*I*, Figs. 57 and 58) is placed. This wheel moves up and down the axial tube on a screw-thread, cut twenty-five threads to the inch. A complete revolution of the wheel, therefore, raises or lowers it a millimeter. The margin of the wheel is divided into fifty spaces, each of which therefore represents twenty microns. A pointer (*N*, Fig. 57) serves to indicate the number of spaces passed in a partial revolution of the wheel, and thus to show the thickness of the sections cut.

The knife stage (*F-B*, Figs. 57 and 58) consists of a tubal base (*F*), which surrounds an axial tube and rests on the movable wheel; and of two flanges (*B*), which extend above the freezing stage on each side for the support of the cutting blade.

The base of the knife stage is moved up the axial tube by screwing the wheel upward. It is forced down the axial tube by the spring (*E*, Figs. 57 and 58) whenever the wheel is turned so as to be carried downward. The flanges of the knife stage support parallel glass tracks, upon which the cutting blade is carried to and fro.

For cutting sections a razor or a plane, or almost any good steel blade with a straight edge, may be used.

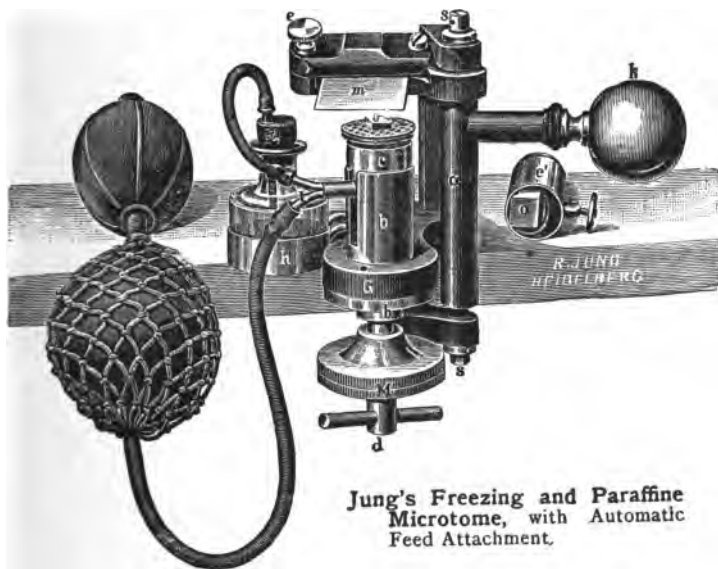


FIG. 59.

Jung's freezing and paraffin microtome.—This instrument is used by workers in this country and in Europe. It has a freezing and automatic feeding attachment, and is adjustable to any desired thickness from five microns upward. The knife *m* is clamped to the vertical shaft *a* by means of the screw *e*. The lever *k* moves the knife over the object *o*. The illustration (Fig. 59) shows the freezing attachment in place. By pressing the rubber bulb the carbon dioxide is transmitted from the bottle *h*

into the cylinder *c*, and by rapid evaporation the heat is extracted from the object, which soon freezes to the proper consistency for cutting into sections. This freezing attachment may be separated from the microtome and the cylinder *c'* substituted if sections are to be made of objects not requiring freezing.

Figure 60 illustrates the apparatus which is applicable to the microtomes shown in Figs. 30 to 35, when freezing is resorted to for cutting sections. The attachment *A* is a cylindrical box upon which the specimen to be frozen is placed. The spray of

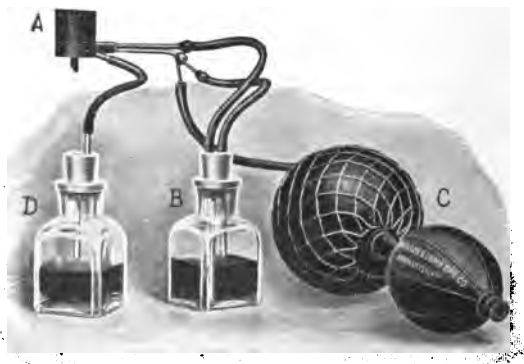


FIG. 60. — Ether or Rhizolene Freezing Attachment.

ether or rhizolene is projected against this stage by means of the atomizer *C*. The excess of fluid is caught by the bottle *D*.

Orienting. — In orientation the sections are made in definite planes in relation to certain points so that the operator is able to determine the exact plane, the direction the microtome knife has travelled, and the precise thickness of the sections. If it is desired to reconstruct the object which has been sectioned, it may be so accomplished if the orientation has been carefully done. There are several methods for orientation adopted by microscopists, but the description of two will suffice in this connection to give the student a conception of what the general method is.

Woodworth's method for orienting small objects. — In preparing small objects for the microtome, Mr. W. McM. Woodworth, of Harvard University, found some difficulty in orienting the specimens so that he could obtain sections in definite planes, until he developed the following plan. He took paper with raised parallel lines, or a grained or rep surface, such as is usual with linen cloth writing paper, and cut rectangular strips 5×15 mm., so that the cut edges were parallel and perpendicular to the raised lines on the surface of the paper. He glued the smooth side of the paper to a slip of glass by means of gum arabic; after drying, the exposed or line side of the paper was covered with a thin coating of gum arabic solution, applied with a brush. And after this second coat dried, he placed on it a thin coating of celloidin, made of flexible collodion diluted with three parts of ether. Apply this collodion just before using the mount for orienting the object, because if applied some time before using it will crack and fall off. The object to be oriented, previously soaked in turpentine, is drained of the surplus turpentine by means of blotting-paper, and then arranged on the surface of the collodionized paper, where it will stick if the oil has been well drained off. The axes of the object are arranged with the desired relation to the raised lines on the paper. When thus suitably oriented the glass slide is exposed under a bell-glass to the ether vapor for a few seconds; this will soften the collodion, to which, when it dries again, the object will be found firmly attached in the desired position. Now put a drop of turpentine on the object, and the mount is ready for the paraffin bath. After soaking for a time in the paraffin, the metal box is placed around the glass slip, and the object is imbedded in paraffin. When the paraffin cools, carefully remove the metal box and trim the block so that the edges of the paper show all around. Place in a vessel of water, and the gum arabic will be dissolved, and the paper may be stripped off, leaving the paraffin block marked with the raised lines, and the object resting just below them with the collodion film between the object and the delicate lines. These lines will now permit the correct orientation of the block in the microtome

and render it possible to section the object along certain known planes. (*Bull. Mus. Comp. Zool.*, XXV, pp. 45-47.)

Eyclesheimer's method of orienting.—The metal box used for imbedding is perforated with a number of small holes along the sides and ends; through these are run silk threads, and fastened on the outsides of the box by means of celloidin. The object is oriented on these silk threads, and the imbedding mass is poured in and hardened. After the hardening has been completed, the ends of the threads are detached by a drop of ether, and the ends hanging on one side and on one end of the box are blackened with lampblack mixed with celloidin. The other ends of the threads are now pulled through the box, and the lampblack leaves distinct lines through the imbedding mass which serve to orient the block and its object on the microtome.

Fixing the sections to the glass slips.—After the sections have been properly cut, with or without the imbedding mass, described in previous pages, and it is proposed to mount them on glass slips for permanent preservation, it is necessary to resort to some method for fastening or fixing them on the glass, so that they will not change their positions during the treatment in staining and in mounting.

If the specimen was stained in bulk, then mounted or imbedded in paraffin and the sections cut with the imbedding mass attached, the following methods are recommended as giving the most satisfactory results in causing the sections to adhere to the glass slip while the imbedding mass is being removed, and during the progress of other manipulations necessary to complete the mount:—

MAYER'S ALBUMEN FIXATIVE:—

White of egg	50 cc.
Glycerin	50 cc.
Salicylate of soda	1 gramme

The method for using this fixative is as follows: A small drop of the albumen is placed on the slip, or the cover-glass, and, by means of a clean finger, spread over the place where the section is

to be mounted until a very thin film of the albumen is secured, — the thinner the better. Arrange the sections in the order in which they are to remain, and place the glass slip under a bell-glass for several hours until the water has evaporated. Warm for a few minutes in the water-bath, and as the paraffin is melted the albumen is carried away with it and the sections are left firmly adhering to the glass. The tissues may now be stained and dehydrated without danger of displacing the sections. The author in using this method prefers to attach the sections to the cover-glass, because so little space is occupied by the cover-glasses; and, if the slips have rings spun on them, there is difficulty of destroying these rings while staining and dehydrating the sections after they are attached to the glass slip.

If the sections are found to be inclined to fold, the method of procedure is as follows: After preparing the glass with the albumen mixture, place on it a drop of water and spread over the surface, arrange the sections, and warm the slide until the sections open and flatten out. Evaporate the water at a temperature below the melting point of paraffin, then melt the paraffin and transfer to a vessel containing benzol or xylol, which will dissolve all the paraffin remaining. This will generally take an hour or two.

Huber's method (*Jour. App. Mic.*, Vol. I, p. 103). This method is conducted with the sections on the cover-glass. These glasses must be carefully cleaned before they are used for this purpose. This is accomplished by placing them in sulphuric acid, rinsing, transferring to vessel containing acetic acid, and after remaining in this acid a few minutes the cover-glasses are rinsed again and then placed in a vessel containing alcohol. Wipe them dry with a clean soft cloth, and they are ready for the process. Heat a porcelain dish containing water, with several of the paraffin sections floating on the water, until the sections just begin to unfold, remove the flame, taking care not to heat sufficiently to cause the paraffin to melt. When these sections are flattened out, place the others to be mounted in the water until they also are flattened out. In the meantime coat

the cover-glasses with the albumen fixative as directed above. Grasp one of these glasses in the forceps and pass it under one of the flattened-out sections on the water, gently lift the glass up under the section until it comes in contact with the albumen surface, and then with the assistance of the section lifter, or a needle, hold the section down in contact with the surface of the glass and draw from the water. Drain the water off by means of filter-paper or a blotter. Place the cover-glass aside until the water evaporates, from five to eight hours; or heat in the water-bath with a temperature not above 40° C. After the water has evaporated hold the cover-glass over a flame until the paraffin is melted, care being taken not to overheat, and then transfer to a vessel containing xylol until all of the paraffin is dissolved, which will take two or three minutes. Drain off the xylol and treat sections with alcohol to remove the xylol, and stain or clarify and mount in balsam.

Eisen's method (*Zeitschr. f. wiss. Mikr.*, Bd. XVI):—

The basis of this method is alcohol in the place of the albumen given in the above methods. The slide is flooded with 70 to 85 per cent alcohol, and the sections are arranged on it. Hold over a flame until the sections flatten out, but taking care not to melt the paraffin. Drain off the alcohol by means of filter-paper or blotter. Place on the slip a piece of smooth blotting-paper, the size of the glass, which has been saturated with alcohol the same strength as that used for flattening out the sections. Put on top of this blotter another dry blotter, and press them well in contact with the glass. The object of this is to cause the sections to adhere in close contact with the glass. Remove the blotters, take off all lint, and dry at a temperature not above 40° C. Dissolve the paraffin as given in former methods. It is claimed that this method will cause the sections to attach firmly to the glass, and the time consumed in completing the work is much shorter than that required in the albumen process.

Koninski's method (*Zeitschr. f. wiss. Mikr.*, XV, p. 161, 1898):

1. Cover slide with film of gelatin.
2. When gelatin has set, arrange the sections.

3. Smooth down with warm water.
4. Warm plate until gelatin melts, and drain off surplus gelatin with blotter.
5. When dry, place in pure formalin for ten minutes.

The section is now so firmly attached that "boiling water will not remove it."

The water method for fixing sections to the slip. — The principle involved in this method is the molecular attraction between the glass and the section. The operation is performed as follows:

Clean the glass slip perfectly by first immersing it in alcohol for some time and then rinsing it with water. Rub the glass thoroughly with a clean cloth until a film of water will stand over the surface uniformly and not in separate drops. If rubbing with the cloth will not accomplish this, then apply with the cloth some finely divided chalk and rub again, wash with water and dry, and if the film still refuses to stand evenly over the surface, reject the glass and try another. The cleaning is very important to a successful completion of the fixing. The sections cut in paraffin, if inclined to roll or bend, are placed on the surface of warm water until they unroll, but care must be exercised not to heat enough to cause the paraffin to melt. Spread over the glass slip a thin film of water and place the sections on it, apply a gentle heat, below the melting point of the paraffin, until the water is entirely dried out, and the sections will be left in close contact with the glass, so that the after treatment with the staining and other solutions will not remove it. The paraffin may be removed by dissolving in a tube containing xylol. Do not place the glass slips containing the sections in solutions which are alkaline in reaction, because alkaline fluids will detach the sections. To be successful with this method there must be a continuous surface in contact with the glass, and the surface of the glass must be well cleaned. The larger the sections and the thinner they are, the more perfect will be the results.

Sections which are cut in celloidin or collodion imbedding masses must be fixed to the slip by means of Summer's ether-vapor method. The sections are first placed in 95 per cent

alcohol for a few minutes, and then arranged on the slip; sulphuric ether *vapor* is poured on the slip from a bottle half full of the liquid ether. The celloidin will become softened and transparent. The slip is now transferred to a vessel containing 95 per cent alcohol, when it will be found that the sections are firmly fastened to the glass.¹

Weigert's collodion method (Lee's "Vade-Mecum," p. 221; also *Zeitschr. f. wiss. Mikr.*, p. 490, 1885):—

Wet the knife with alcohol during the cutting of sections. Soak a piece of porous paper with alcohol and bring it in contact with the section on the knife, and then by a horizontal, upward motion detach the section from the knife; it will remain in contact with the paper if carefully worked. Collodionize the glass slips by pouring on them collodion and spreading to a thin film. Take one of these collodionized slips and bring the paper in contact with it, with the section down on the surface of the glass; press gently until the section is brought in connection with the glass and then remove the paper. Remove any excess of alcohol by means of blotting-paper, and cover the plate with collodion and place in eighty per cent alcohol until wanted for staining or other manipulation.

¹ *American Monthly Microscopical Journal*, p. 73, 1887.

CHAPTER VI

STAINS, THEIR PREPARATION AND USE

Stains.—The staining of tissue is resorted to in order that the groups of cells in the object under examination may be clearly marked and thus become distinctive. Without this method of staining the sections it would be very difficult, in some instances, to distinguish the minute part sought for from the rest of the tissue surrounding it.

The requisites for a good stain are :—

1. When in full strength its action should be *intense*.
2. It should have *selective* properties rather than *diffusive*.
3. It should penetrate well all portions of the specimen, staining equally the similar tissue near the surface and toward the centre of the section.
4. The color should be permanent.
5. It should not disintegrate the specimen, even though the process be prolonged.

All stains may be divided into two groups :—

1. General stains.
2. Selective stains.
 - (a) Nuclear stains.
 - (b) Cytoplasm or nucleoplasm stains.
 - (c) Cell-development stains.

General stains act on all parts of the subject alike, while selective stains pick out one or more groups of fibre in the section, and, when properly applied, leave all other systems of the section unaffected.

To produce the best results with the selective stains, two methods have been proposed :—

1. The staining must be stopped just as soon as the parts to

be colored have received the maximum degree of dye and the rest of the section is beginning to be affected.

2. The section may be left in the selective stain and continued much beyond the first step, and the extra color eliminated by washing in alcohol, or dilute hydrochloric acid, in which the color stain is soluble. This is known as Flemming's method, and it is only applicable to staining *sections* and must not be used for staining *in toto*.

Ehrlich divides the anilin dyes into : —

1. Acid.
2. Basic.

The first is subdivided into :

1. Eosin and fluorescin.
2. Picric acid and aurantia.
3. Tropæolin.
4. Alizarin purpurin, rosol acid.

The second group is subdivided into : —

Fuchsin, methyl-violet, gentian violet, methylene-blue, vesuvin, Bismarck brown.

The following single stains are recommended for general use in the laboratory : —

Anilin-green. — This is a delicate nuclear stain and stains the nucleoli in brilliant color. Stain in saturated solutions and wash out with strong alcohol. Clear with cedar or bergamot oil.

Bismarck brown. — This is also a nuclear stain. Dissolve in alcohol. This stain is especially valuable for mass-staining, and although it acts very rapidly, it never overstains. It is permanent. For protoplasm, connective tissue, bacteria, and living organisms Bismarck brown gives good results. If used in staining sections, the indirect method should be adopted. Wash after staining in absolute alcohol.

Carmine. — There are a number of carmine solutions differing in the other ingredients combined with the carmine. Such, for instance, as ammonia-carmine (Gerlach's formula), alum-carmine (Grenacher's formula), borax-carmine (Grenacher's for-

mula), picro-carmin (Ranvier's formula), Mayer's carmalum, or alum-carmin. Carmalum is one of the very best of the carmin dyes for staining bacteria, and for other tissues, and it is therefore given with a strong recommendation for those purposes for which it was developed. The formula is as follows : —

Carminic acid	1 gramme
Alum	10 grammes
Distilled water	200 cc.

Dissolve with heat and filter.

Add a few crystals of thymol to preserve the stain.

After staining wash out with water.

Dahlia or *Hoffman's violet*. — Dissolve in water and use either neutral or acidified with acetic acid. Wash out with strong alcohol. Professor A. B. Aubert ("The Microscope," XI, p. 270) recommends the following formula, which is suitable for nuclear and fresh tissue stains : —

Glacial acetic acid	12.5 cc.
Absolute alcohol	50 cc.
Water	100 cc.
Dahlia nearly to saturation.	

This formula will stain in twelve hours or less.

Eosin. — Stains protoplasm deeply, muscles, nuclei. Also sieve tubes. This stain is especially valuable as a contrast stain ; for this purpose, see compound staining.

Gentian violet. — This stain is one of the most important single stains and is also much used in compound staining. It is especially valuable for staining living tissue and has the property of coloring living cells without destroying their vitality. It is soluble in either water or in alcohol. Ehrlich's formula is probably the best form for this stain : —

Gentian violet	1 gramme
Anilin oil	3 grammes
Alcohol	15 grammes
Water	80 grammes

Tissues will stain in this within twenty-four hours, at ordinary temperatures, but by raising the temperature the time may be much shortened. After staining wash in alcohol, then place in chromic acid, and then again in alcohol to dehydrate, and clarify in oil of cloves, and mount in balsam.

This stain is useful for bacteria, decolorized chlorophyll bodies.

Methyl-green. — Dissolve in water and add a little acetic acid (0.75 per cent). A standard stain, and is valuable for coloring nuclei of fresh tissue and nerves. Wash with water slightly acidified. Complete fixation is accomplished by the after use of the vapor of osmium and Ripart's and Petit's solution, viz.: —

Copper chloride	0.39 grammes
Copper acetate	0.30 grammes
Acetic acid crystals	1 gramme
Camphor water	75 grammes
Distilled water	75 grammes

The action of the osmium is to strengthen the fixing properties of the solution.

Safranin. — Dissolve in water and alcohol, viz.: —

Safranin	1 part
Absolute alcohol	100 parts
Water	200 parts

(A. B. AUBERT.)

An important stain for nuclei, bone, and connective tissue.

Wash with absolute alcohol after staining.

Victoria blue. — Make a saturated solution of this stain in water. Wash after staining in alcohol and clear in oil of cloves (Lee). This is a nuclear stain and gives beautiful results and is easy to manage. Lee says that the stain has a strong affinity for elastic tissue, and for this use it must be dissolved in alcohol diluted with two or four parts of water.

In the process of coloring objects it is necessary, in order to get the best results, that the tissue should be dead, because living tissue will not, as a general rule, take up the stains so

readily, for the cells seem to repel the coloring matter. It has been found, therefore, advisable first to fix, or artificially kill, the specimen before the sections have been cut; this is particularly true in reference to animal subjects, and in the case of portions of plants where the centres of life will totally change unless they are quickly fixed in the form naturally assumed during the living period.

For a detailed account of the processes for killing and hardening tissues, see page 71.

How to stain. — Determine first what portion of the object you wish to bring out for study, and then select the character of stain which has an affinity for that tissue, and then proceed as follows : —



FIG. 61. — Naples Staining-jar.

Dissolve some of the dry stain in alcohol, or water, depending on the condition of the object and the character of the stain, and place the solution in a Naples staining-jar (Fig. 61), if the sections are fixed to the slips; if, however, the sections are fixed to the

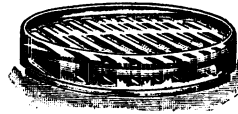


FIG. 62. — Moore's Staining-dish.

cover-glasses, it will be best to use a dish something like Moore's staining-dish (Fig. 62), which has parallel ridges for holding the cover-glasses, and a cover-dish for keeping out dust. This dish is so constructed that it may be used for holding slides also.

Now having fixed the section securely to the slip or the cover-glass, by one of the methods described above, and having dissolved the paraffin used in imbedding, place the section and its slip in alcohol for a short time, of strength of 95 per cent, and then transfer to the staining-dish, if the stain was dissolved in alcohol, and permit the slip to remain in this solution until the section is fully colored to the intensity desired. The length of time will depend on the character of the section and

the stain used. These points can be determined by reference to the detailed account given of the stain elsewhere in this book. If the stain was dissolved in water, it will be necessary to transfer the section and the slip from the 95 per cent alcohol to a vessel containing water and allowed to remain for a few minutes and then placed in the vessel containing the stain. The general rule for transferring from alcohol to water is to pass the section first through 75 and 50 per cent alcohol, and then to water, to prevent injury to the delicate cells of the specimen; but some American authorities claim that this is not necessary if a very large amount of water is used, for the alcohol is taken out so quickly, there is no time for the alcohol and water to mix in the section, and thus diffusion currents are avoided.

After the stain has penetrated long enough to give the desired intensity, the extra color is washed off the section with alcohol or water (depending, of course, upon which solution of the stain is used), for two or three minutes, and then dehydrated in 95 per cent alcohol. The dehydrating will take from a few minutes to several hours. (See Dehydrating.)

Double or compound staining. — Beautiful results are secured by this process. The object of double staining is so to differentiate the tissues as to bring out in striking contrast each group of fibre in the section and thus facilitate the study of them under the microscope. Double staining is accomplished by the use of two dyes, one to stain the nucleus and the other to color the protoplasm around the nucleus or the cell walls surrounding. The method is the same as that used in simple staining, and the following represent some of the most important combinations for this purpose:—

1. Carmine-anilin blue. Clear, with turpentine and mount in balsam. Suitable for all kinds of tissue and nerve centres. When staining in mass use in dilute condition.

2. Hæmatoxylin-eosin. The sections should be very thoroughly washed after staining in the eosin and before transferring to the hæmatoxylin, since the presence of the former will precipitate the latter. Stains embryological sections.

3. Safranin-hæmatoxylin. This combination gives beautiful results. Stain first with hæmatoxylin, very slightly, then wash out with water followed by alcohol acidulated with hydrochloric acid, and stain for some hours with safranin. Stains nuclei cytoplasm beautifully.

4. Safranin-gentian.
5. Gentian-violet eosin.
6. Methyl-violet eosin.
7. Eosin and methyl-green.
8. Bismarck brown and methyl-green.
9. Dahlia and eosin.

Resegotti has found by experiment that those stains which do not select the nucleus will wash out from the fibres the stains which are purely nuclear colors. He has classified them, therefore, into the following groups:—

Nuclear stains.—Safranin, dahlia, methyl-violet, gentian violet, magenta and Victoria blue. These are washed out by the stains mentioned in the next paragraph.

The secondary or non-nuclear stains are methyl-green, iodine green, Congo, orange, acid fuchsin, cyanin, eosin, methyl-eosin, bordeaux, and vesuvin.

It will be of importance to note that the properties possessed by the last group to wash out those found in the first list will enable the student to procure beautiful contrasts by the chemical decoloration one of these colors produces on the other. It must be borne in mind, however, that the washing-out process must not be prolonged, otherwise the color in the nucleus will be attacked and the double staining will be destroyed. Transferring the sections to water will stop the elimination of the color; or they may be cleared and mounted at once. Clove oil may be used for the clearing agent, but it will also extract some more of the color, and this fact must be remembered or the section will present a faint contrast when mounted. Cedar oil, xylol, and bergamot oil do not attack the stain, and they may be used instead of the clove oil.

In the *Journal of the Royal Microscopical Society* for Octo-

ber, 1893, the following process for double-staining vegetable tissue is given:—

The sections are first decolored by eau-de-Javelle (hypochlorite of potash), and then left for a quarter of an hour in a concentrated alcoholic solution of cyanin, then washed with absolute alcohol and placed for a quarter of an hour in a 5 per cent ammoniacal solution of Congo red. After washing in alcohol and mounting in xylol Canada balsam, the sections present a magnificent double-staining; the liquefied membranes are colored an intense blue, and the cellulose membranes are dyed rose color or red.

To stain in bulk the following stains have been found suitable: carmine (borax and alum), cochineal, hæmatoxylin, Mayer's para-carmine, Grenacher's borax-carmine (concentrated solution of carmine, 2 to 3 per cent borax diluted with equal bulk of alcohol of 70 per cent), Mayer's carmalum.

Hæmatoxylin is one of the best stains for staining in bulk, particularly with those tissues which have been treated with osmic and chromic solutions. It is, however, very difficult to make solutions of this stain which will keep for any great length of time. This makes the staining properties of hæmatoxylin very uncertain unless fresh solutions are used each time. In accordance with the experiments conducted by Mayer, he has found that it is best to use hæmatein instead of hæmatoxylin, which avoids the tedious method formerly used in "ripening" the hæmatoxylin, because the hæmatein is already in a ripened condition. Because of the difficulty at times in securing the hæmatein in a sufficiently pure form, Lee recommends the use of hæmatein-ammonia. This latter stain can be purchased from dealers in dyes. The preparation of Mayer's solution is as follows:—

MAYER'S HÆMALUM:—

Hæmatein or hæmatein-ammonia	. . .	1 gramme
Alcohol 90 per cent	. . .	50 cc.

Dissolve with heat and add to solution of 50 grammes of alum in a litre of water. Filter and add a crystal of thymol to preserve the stain.

The action of this stain is quick, and specimens which are placed in it when it is in a concentrated condition are stained in a few moments. It acts well either in the concentrated or diluted form. It is highly indorsed for staining in bulk. Wash out with water after the staining is completed. In clearing do not use oil of cloves, but clear with oil of bergamot, and then remove this oil with oil of turpentine.

DELAFIELD'S HÆMATOXYLIN STAIN:—

Hæmatoxylin crystals		4 grammes
Absolute alcohol		25 cc.
Ammonium alum		52 grammes
Distilled water		400 cc.
Glycerin		100 cc.
Methyl alcohol		100 cc.

Dissolve the hæmatoxylin in the absolute alcohol, the alum in hot water, and then add the hæmatoxylin solution drop by drop to the alum solution after it cools. Expose to the action of the light and air for three or four days until the liquid turns dark, filter and add the glycerin and methyl alcohol. Keep in a tightly stoppered bottle. For staining, dilute with five to ten volumes of water.

It is better to allow this stain to ripen for a month or more before using. It will keep for years and is a very strong stain. It is excellent for embryos and tissues *en masse*. It forms a good combination with safranin, the hæmatoxylin staining the cellulose a rich purple, while the safranin gives to the lignified tissues a red color.

This solution is also a stain for chromatin, and it is sometimes used for staining nuclei.

EHRLICH'S HÆMATOXYLIN STAIN:—

Hæmatoxylin		2 grammes
Absolute alcohol		100 cc.
Distilled water		100 cc.
Acetic acid (glacial)		10 cc.
Glycerin		100 cc.
Alum in excess		

Ripen in the air until the solution turns dark. Keep in well-stoppered bottle. It retains its properties for years. It stains in bulk, and there is no danger of overstaining. It acts with great rapidity on sections, staining them in a few minutes a deep color. This stain has great affinity for chromatin.

Heidenhain's iron hæmatoxylin stain. — There are two solutions comprising this stain, and they must be kept separate. The first consists of a 1.5 per cent solution of ferric alum or ammonia sulphate of iron. Care must be exercised to use the double salt of the sesquioxide of iron and not the protoxide. The crystals must have a clear violet-color. The second solution is made by dissolving hæmatoxylin in water, a 0.5 per cent solution.

To stain, first treat the specimens in the ferric-alum liquid for a half-hour to two or three hours. Wash with water. The ferric-alum is not the stain but is simply used as a mordant to prepare the specimens for the second solution, which is the stain. After washing, place the tissue in the second solution for a half-hour and wash, and again treat with the mordant, which washes out the stain slowly, until the desired differentiation is obtained. The changes must be watched under the microscope. A short immersion in the hæmatoxylin will produce blue preparations, while a prolongation of the staining will give black colorations. The blue color brings out strongly the nucleus, and the cytoplasmic tissues are shown by lighter shades. The black color obtained by long staining shows clearly the chromosomes, the central corpuscles staining deep black, red corpuscles black, while the cytoplasm will generally remain colorless, or will turn gray.

This stain is to be used only with sections which must be very thin. After staining wash with water, then pass through several changes of alcohol of varying strengths, clear in xylol, and mount in balsam.

Weigert's method for staining the central nervous system with hæmatoxylin.¹ — The specimen to be stained must first be hard-

¹ Dr. B. Pollack in *Arch. f. Mikr. Anat.* (also see *Jour. Roy. Mic. Soc.*, p. 837, 1897) "calls attention to the importance of Weigert's painstaking work on the human

ened in Müller's fluid, or Erlicki's solution, or in a preparation of formaldehyde. This hardening process takes from twenty-four hours to ten days, depending upon which method is adopted for the purpose. (See chapter on hardening solutions and methods.) The tissue is then transferred to grades of alcohol *without washing*. Imbed in celloidin, which hardens in 80 per cent alcohol. The celloidin block is then mordanted in a water solution containing neutral acetate of copper, 5 per cent; acetic acid, 5 per cent; and $2\frac{1}{2}$ per cent chrome alum. Make this solution by adding the chrome alum to hot water and then in succession the acetate of copper and the acetic acid. The mordanting must continue for four or five days. Wash in alcohol, cut the sections, and then stain in the following:—

1. Hæmatoxylin crystals	1 gramme
Absolute alcohol	10 cc.
2. Lithium carbonate	1.5 grammes
Water	100 cc.

For use mix just before needed. Continue straining action for two to twenty-four hours, the duration depending on the character of the tissue. After the stain has acted long enough, wash off the excess of the solution and transfer the tissue to the following decolorizer:—

Potassium ferricyanide	2.5 grammes
Borax	2 grammes
Water	100 cc.

When this decolorizer has acted long enough, which is indicated by the blue-black condition of the medullated nerve-fibres

neuroglia. Golgi's method is applicable to animals almost exclusively, and especially to embryos; Weigert's method is applicable to man. Golgi's method colors only some cells; Weigert's colors all nuclei and fibres. Golgi's method yields a silhouette; Weigert's shows the normal form and size. Golgi's method distinguishes only bells with processes; Weigert's method shows cell body, or nucleus differentiated from the processes. Golgi's method colors besides the neuroglia the nervous tissues; Weigert's method differentiates the neuroglia—a fact which in part explains how Golgi, Caljal, and most anatomists hold the neuroglia to be nervous, which Weigert, like Ranvier, vigorously denies."

and the yellow tint given the gray matter, the tissue must be washed for some time in running water, dehydrated, cleared in oil of cloves, and mounted in balsam.

This method is not suitable for staining *in toto* because the hæmatoxylin will not penetrate.

Golgi's bichromate and nitrate of silver method for staining the nervous system (rapid method).— A hardening mixture is first used in which the nervous tissue, in small portions, is soaked for about two days in the dark. The specimens should be small and the quantity of the hardening solution liberal in volume. Care must be taken neither to over nor under harden, for in the former case there will be no impregnation of the silver, and in the latter condition the silver will diffuse too much. The character of the animal must determine the length of time in which the tissues are subjected to the action of the hardening solution.

HARDENING FLUID: —

Bichromate of potassium of 2 to 2.5 per cent strength	8 parts
Osmic acid of 1 per cent strength	2 parts

After hardening as above indicated, the specimens are brought into a weak, or used, solution of silver, so that precipitation will not occur when the full strength of the fluid is used. Wash until precipitates cease, and then pour on a liberal quantity of the following impregnation solution:—

Silver nitrate crystals	0.75 grammes
Distilled water	100 cc.

In winter perform the impregnation in a warm place, and permit the specimens to soak for a period of 24 to 48 hours. The length of time is not so important after the first 24 hours; specimens may remain in the liquid for several days without harm, provided the solution is renewed.

The specimens are now washed in 95 per cent alcohol, and the washing is repeated until all of the free nitrate of silver is washed out. The alcohol must remain transparent after the specimens have been in it for two or three days. The sections

are now cut by the celloidin method. Place the celloidin blocks in absolute alcohol, changing several times, clear with creosote for a few minutes, then in oil of bergamot or oil of turpentine for fifteen minutes. Or the clearing mixture may consist of carbolic acid, oil of bergamot, and oil of cedar wood, equal parts, in which the clearing is accomplished rapidly. Cut the sections and mount in damar *without* the cover-glass, for it has been found that those sections which are mounted under cover-glasses lose their properties and soon ruin. The drying of the damar should be hastened by means of heat.

Golgi's method stains axis-cylinders, ganglion cells, and neuroglia cells in the order given. In the use of the stain sometimes troublesome precipitates form at the surface, which are injurious to the clearness of the image. To avoid this Sehrwald used gelatin in the following manner: The specimen was placed in a paper box containing a 10 per cent solution of gelatin and water before impregnating with silver. A gentle heat was applied to the gelatin until it was melted and the specimen became well immersed. The mass was then brought into the impregnating solution, and after the silvering the gelatin was removed by means of warm water saturated with chromate of silver.

According to Dr. G. C. Huber¹ permanent preparations of nervous tissue stained by Golgi's method may be obtained in the following manner:—

The pieces of the nervous tissue are to be hardened and silvered according to the procedure advised by Ramon y Cajal² and von Kölliker, and celloidin sections cut under 95 per cent alcohol. The sections are then immersed for fifteen minutes in creosote and then for some minutes in turpentine. They are now spread out on a slide, pressed down with filter-paper, and covered with turpentine balsam. The slide is then gradually and carefully heated over a flame until the balsam will become hard when cooled. At this stage the cover-glass is put on the hot balsam. The heating takes from three to five minutes.

¹ *Anat. Anzeig.*, VII, p. 587, 1892; *Jour. Roy. Mic. Soc.*, p. 707, 1892.

² Lee's "Vade-Mecum," 5th ed., p. 418.

Dr. Kopsch¹ suggests a modification of Golgi's method as follows: The fixative is a mixture of 40 cc. of a 3.5 per cent solution of potassium bichromate and 10 cc. of formalin. The specimens are placed in this fluid for twenty-four hours, and then in a pure solution of bichromate, where they are allowed to remain two or three days. The specimens are then transferred to 0.75 per cent silver nitrate solution for three to six days. Nervous tissue thus stained does not suffer from precipitates.

When carmine is used for staining *en masse*, the washing out must be performed with a weak solution of hydrochloric acid (about 0.1 per cent).

Mayer's carmalum, or alum-carmine, is prepared as follows:—

Carminic acid	1 gramme
Alum	10 grammes
Distilled water	200 cc.

Dissolve with heat and filter.

Add a few crystals of thymol to preserve.

Wash out with water. Before staining in bulk see that the specimens are not alkaline, because if so the stain will not act well. This is an excellent stain.

Gram's method for staining bacteria.—By this method the bacteria are stained while the enclosing tissues remain unstained. The bacteria are stained a dark blue, and they are brought out very clearly from the surrounding tissues.

The dyes which are suitable for this method are the anilin colors, especially gentian violet and fuchsin.

The original plan devised by Gram was first to stain in Ehrlich's anilin water gentian violet and then treat with iodine. The anilin-water, gentian-violet solution is prepared as follows:

Make a saturated solution of anilin oil with water and filter. Add to this a saturated solution of gentian violet in alcohol until a dark color is obtained; it will take about 1 cc. of the gentian solution to 20 cc. of the anilin water to accomplish this. Or we may prepare the stain as follows:—

¹ *Anat. Anzeig.*, XI, p. 727, 1896.

Anilin oil	3 parts
Water	80 parts
Gentian violet	1 part
Alcohol	15 parts

The specimens of bacteria fixed to the cover-glass are first placed in absolute alcohol, and then transferred to the stain for a few minutes, where they become deeply stained. The cover-glass is now placed in a weak solution of iodine and iodide of potassium, prepared as follows:—

Iodine	1 gramme
Iodide of potassium	2 grammes
Water	300 grammes

The section remains in this solution from one to three minutes, and is then placed in absolute alcohol until nearly all of the color is washed out; in fact, the section seems to have become decolorized entirely. Clarify in clove oil and mount. Under the microscope the bacteria will be seen colored a beautiful dark blue and the nuclei without stain. The nuclei, however, may be also stained by following the above method with Bismarck brown, thus giving the nuclei a shade of yellow or brown, with the bacteria still a dark blue and very distinct.

It is unfortunate that all bacteria will not stain under the Gram method with the same degree of success, but so many of them yield to this treatment that it has become one of the standard methods of staining among bacteriologists.

“Intra vitam” staining.—This term is used to apply to the staining of living tissue, without destroying the life of the organism. The method is of great advantage in determining certain physiological conditions that would be impossible of satisfactory solution without the use of this method. The stain must be considerably diluted, however, and feebly alkaline or neutral. The strength of the stain ranges from 1:10,000 to 1:100,000, depending upon the character of the animal to be treated and the tissue to be stained. It is best to experiment by starting with a strength of 1:100,000, and increasing the coloring matter until the desired strength

is secured. If the animal is aquatic in habits, the staining can be accomplished by adding methylene-blue to the water in which the animal is living. Certain of the tissues are colored by this method, such as cytoplasm of some cells, sensory nerves, and lymph spaces. Marine animals are also satisfactorily examined under this method by simply adding the methylene-blue to the sea-water in which the animal is living, and during its life the coloring matter is taken into its system. It is important, however, in this class of work to note just when the special tissue is stained its maximum, because the animal begins to throw off the stain after a certain period and soon leaves the tissues in their normal condition.

In some cases it is necessary to inject the staining solution into the living animal to secure the desired results. The injection is made into the vascular system or the body cavity of the living animal, and as soon as the coloring matter has had time to penetrate the organs and stain the tissues under study, these tissues are extracted and mounted for preservation.

The following stains have been found to yield good results by the *intra vitam* method: Methylene-blue, Congo red, Bismarck brown, cyanin, dahlia, gentian neutral red, methyl-violet, safranin, and malachite-green. Methylene-blue gives the best results on the sensory nerves; for Infusoria use Bismarck brown; and for cell tissues use Congo red, methylene-blue, gentian violet, and dahlia. The methylene-blue stain is specially valuable in the investigations of the tannin vesicles; the vesicles are separated from the other tissue with a distinct deep blue color.

To fix the stain for preservation transfer to a concentrated aqueous solution of picrate of ammonia for one-half hour to twenty-four hours, and mount in pure glycerin (Dogiel). In order to prevent the macerating effects produced by the picrate of ammonia on some tissue, there is added to the fixing-bath a 1 per cent osmic acid solution at the rate of 1 to 2 per cent of the bath. Feist has claimed that if picro-carmin be used, the blue color is well preserved, provided the action of the picro-carmin is not allowed to continue for a long period.

Staining *intra vitam* may be conducted with much interest to the investigator by placing the living forms, if minute, on a glass slip and covering with a suitable size cover-glass resting on bits of wax. Permit the diluted color to flow gently through the space between the glasses, and watch the results through the microscope. When the desired tint is obtained in the tissues of the animal, discontinue the flow of the coloring fluid and permanently fix and mount.

It has been discovered by Herr A. M. Przesmycki¹ that different organisms act differently in *intra vitam* staining; some organs even in the same animal exhibit less receptivity than is shown by others. He also proves that the parts colored *intra vitam* lose their color after death, and when the organism is transferred to fresh water the stain is washed out.

Paul Mayer² likewise proves that Bismarck brown dissolved in sea-water and Caprellidæ placed in the solution, the contents of the stomach and the hepatopancreas were stained brown. Transferred to pure sea-water the specimens lost their coloring in a few hours, and the animals seemed to be unharmed.

Professor P. Ehrlich,³ one of the earlier investigators on *intra vitam* staining, gives the following items concerning vital infusion of nerves with methylene-blue: "There are two conditions necessary to get the methylene-blue reaction:

"1. Saturation with oxygen.

"2. Alkaline reaction.

"The first is obtained by free exposure to the air. To secure the second condition it is necessary to experiment on nerves at rest, since it is well known that when in that condition they are alkaline in reaction. This condition may be obtained by severing the nerves before applying the methylene-blue or by poisoning the animal with curare. The method of procedure is as follows:—

"Inject the vena cutanae magna of a frog with 1 cc. of a

¹ *Biol. Cent.*, XVII, p. 353, 1897.

² *Fauna u. Flora des Golfes von Neapel vi Monographie*, p. 153, 1882.

³ *Deut. Med. Wochenschr.*, No. 4, 1886.

saturated solution of methylene-blue. The tongue and palate are at once colored, but the stain is confined to the blood-vessels, and does not at first affect the nerves. After an hour or two the nerves supplying the taste papillæ appear blue, and meshes of the palate are also stained. The motor nerve-ends show the stain a little later. The color reaction lasts only a short time, often not more than five or ten minutes. It should be fixed at the moment of greatest intensity. If iodine is used for this purpose, proceed as follows:—

“Place the frog in a 1 per cent aqueous solution of potassic iodide, in which metallic iodine has been dissolved to saturation, and inject the blood-vessels with the same solution, thus freeing them from blood as far as possible. Next cut out the parts needed and leave them in the iodine solution from five to twelve hours. Transfer to water, and leave until most of the iodine has been withdrawn from the specimen. As a result of this treatment the nerves will have a dark brown or gray color, and the surrounding tissue will be nearly colorless. Mount in acidified glycerin.”

Examples of how to stain tissues.—The following list of tissues is given for the purpose of furnishing the student some examples of how the stains are used in order to produce the best results in bringing out the details of the section of the specimen under investigation. No attempt has been made to give a complete list of the tissues, but a sufficient number is submitted to enable the student greatly to extend the list after he has familiarized himself with the stains mentioned under each item.

Adenoid tissue.—Delafield's hæmatoxylin method.

Albumen crystals in plants.—Hæmatoxylin produces beautiful violet colors.

Aleurone or protein grains.—Stain with iodine. The treatment with chloral hydrate in 2 parts of water in which a little iodine is placed discolours the chlorophyl bodies, and causes them to swell with the starch grains, and the starch comes out clearly stained blue. Or the chlorophyl bodies may be discolored with alcohol, and then stained with dilute aqueous solution of methyl-violet.

Areolar, adipose, and retiform tissue. — Fuchsin solution mixed with a little hæmalum gives these tissues a deep stain.

Articular cartilage and synovial membranes. — Treat with dilute hæmalum and carmalum, and mount in dilute glycerin.

Axis cylinders and protoplasm nerves. — Either of the following staining methods: Golgi's sublimate, Golgi's bichromate of silver method, methylene-blue method, Weigert's specific neuroglia stain.

Bacteria. — Bismarck brown, safranin, fuchsin, gentian violet, methylene-blue. Spores may be stained by Moeller's method as follows: First place in absolute alcohol for two minutes, then for a minute in a 5 per cent solution of chromic acid; wash and stain in Ziehl's solution for one minute (5 per cent solution in water of carbolic acid, to which is added about one-tenth its volume of saturated solution in alcohol of fuchsin). The stain is heated to boiling. Place the spores in 5 per cent solution of sulphuric acid to decolorize. This will leave the spores red and the germs uncolored. The chromic acid acts on the membranes of the specimen, and thus permits the stain to penetrate to the spores. After fixation the fat cholesterin crystals and other like materials may be separated from the spores by immersion in chloroform for several minutes. Buchner and Hueppe have discovered (1884) that spores do not take the stain so well unless they are heated. This can be accomplished by placing the glass slip or cover-glass on which the spores are fixed in a hot-air oven, and raising the temperature to 120° or 175° C. for one hour.

Bacteria in tissue. — Weigert's or Gram's method. Weigert's method is to dehydrate in anilin oil, wash in water, place on the slide, and remove the excess of the water by means of filter-paper, and then pour on the specimen iodide solution (iodine, 1 part; potassic iodide, 2 parts; water, 300 parts). To decolorize the specimen, remove iodine solution with filter-paper or blotter; dehydrate with a few drops of anilin oil; remove with filter-paper; then treat with xylol to remove all traces of anilin oil; then mount in balsam. The basic anilin dyes take the front rank in staining bacteria.

Blood. — Corpuscles with hæmoglobin stain red in eosin;

methyl-violet stains the nuclei of the corpuscles. Ehrlich-Biondi's method is excellent for this purpose. (To 100 cc. saturated aqueous solution of orange add with agitation 20 cc. saturated aqueous solution acid fuchsin and 50 cc. of a like solution of methyl-green. For the method and its use, see Lee's "Vade-Mecum," 5th ed., p. 215.)

Bone.—Schaffer's method: The bone is first decalcified in nitric acid, and then stained for one-half hour in 0.05 per cent aqueous solution of safranin, washed with water, and then treated for two or three hours in 0.01 per cent solution of corrosive sublimate, and then mounted in glycerin. To make the preparation permanent, wash in alcohol after removal from the sublimate, and clear for a long time in bergamot oil, and then mount in xylol balsam.

Bone and marrow.—One per cent of eosin in alcohol; dilute fuchsin after the bone has been decalcified in nitric acid or in chromic acid; mount in glycerin.

Callus in sieve-plates.—Anilin blue or fuchsin.

Cell contents.—Hæmatoxylin.

Cell granules.—Ehrlich's mixture.

Cell walls.—Methylene-blue; alum-carmine; eosin.

Cellulose tissue.—Aqueous solution of anilin green; the cell walls are stained green, while the walls of parenchymatous cells are stained bluish green. Chlorzinc iodide (Schultze's solution: Dissolve pure zinc in hydrochloric acid, evaporate with a piece of metallic zinc in the solution to a strong consistency, add iodide of potassium to saturation, and finally as much metallic iodine as the solution will take up) added to a vessel of water containing a section of the stem will show in brownish yellow color the cellulose, while the pits remain unstained and thus stand out clear and distinct. The pits are traversed by violet striation, and here and there on them are granulations stained yellow brown. The cuticle also stains a yellowish brown. Grenacher's alum-carmine will yield good results.

Chlorophyl grains.—Methyl-green.

Chromatin in nucleus.—The fixing agents for the nuclei must

invariably be acid in order to preserve the chromatin. Flemming's and Hermann's liquids are best for this purpose. Methyl-green is a good stain for the chromatin, but it sometimes acts very weakly in intensity in different nuclei. Safranin acts well.

Cork cells.—Chloriodide of zinc stains yellow; fuchsin dissolved in alcohol is also good stain.

Cornea.—Methylene-blue method.

Cuticularized layer of epidermis.—Chloriodide of zinc colors this tissue yellow. The true cuticle is uncolored.

Echinoderms.—Methylene-blue method by *intra vitam*.

Elastic tissue.—Weigert's stain colors dark blue, and the other tissues remain lighter color.

Fuchsin	2 grammes
Resorsin	4 grammes
Liquor ferri sesquichlorati	30 cc.
Water distilled	200 cc.

Embryos.—Delafield's hæmatoxylin is excellent for staining small embryos of mammalia. For staining large embryos use carmalum, and also Delafield's acetic-acid-alum-carmines.

Epithelium.—Nitrate of silver; iron hæmatoxylin; methylene-blue.

Fatty oils.—Perosmic acid colors them black or brown.

Ganglia.—Methylene-blue, 100 parts to 1 part of salt water, fix in picrate of ammonia, and mount in glycerin or balsam.

Glands.—Liver is stained in Golgi's rapid method, or by Ehrlich-Biondi's method. The kidney glands are stained with iron hæmatoxylin, and afterward in a solution of fuchsin which brings out the ciliary plateau.

Intercellular bridges and canals.—Use iron hæmatoxylin.

Lignin.—Anilin chloride in dilute solution with addition of hydrochloric acid. The color is pale yellow. Chloriodide of zinc stains a yellow color.

Lymph glands and cells.—Hæmatoxylin and eosin.

Muscles.—Delafield's hæmatoxylin. Eosin.

Nerve fibres, cells, and endings.—Methylene-blue method; Golgi's bichromate of silver method; Weigert's specific neuroglia stain.

Nuclei of cells.—Carmine in dilute potash and mixed with alcohol; ammonia carmine is also generally used, prepared by Hartig's formula.

Nucleus.—Ehrlich-Biondi's stain colors red or orange, and iron hæmatoxylin stains black or gray; Thiersch's borax-carmine; methyl-green; picro-carmine.

Phloëm in vibro-vascular bundles.—Cochineal with acetic acid colors red.

Plasma.—Equal volumes of anilin water and concentrated solution of methyl-violet 6 B. Wash and treat with solution of iodide of potassium, wash and treat with mixture of 1 volume of anilin to 2 volumes of xylol, and then in pure xylol.

Prosenchyma bast tissue.—Cochineal with acetic acid.

Protoplasm, living.—Silver nitrate, in dilute solution, colors the protoplasm dark. Picro-carmine gives a yellow-red color.

Protozoa.—*Intra vitam* method with methyl-green acid solution.

Resin.—Müller's tincture of alcannin gives red, and methyl-violet gives blue colors.

Retina.—Pal's modification of Weigert's method.

Salivary glands.—Carmalum or Heidenhain's hæmatoxylin method.

Sieve-plates.—Anilin blue, place in glycerin, and the latter will extract the color except that which is taken up by the sieve-plates, and they come out in a beautiful color.

Skin.—Hæmalum or carmalum. Treat with picric acid and mount in xylol balsam. Borax-carmine or hæmatoxylin or eosin, and clear in carbol-xylol.

Spinal cord.—Weigert's hæmatoxylin method, and cleared in carbol-xylol.

Spleen.—Hæmatoxylin and eosin, or methylene-blue.

Sponges.—Mayer's tincture of cochineal.

Tannin.—Dilute solution of chloriodide of zinc colors red or violet.

Vascular bundles.—Chloriodide of zinc colors the bast a distinct violet, the lignified parenchyma cells surrounding the bundle are stained red-brown.

CHAPTER VII

MOUNTING THE TISSUE FOR PRESERVATION

Mounting in Canada balsam. — After the sections are properly stained, dehydrated, and fixed to the slip, it becomes necessary to mount them in some medium which has preservative properties; otherwise decay will set in, and in time the sections will be ruined. One of the best mounting media is Canada balsam¹ dissolved in either xylol, benzole, chloroform, or turpentine, because of its refractive properties and the ease with which it is manipulated, and its valuable preserving powers.

In the use of this medium, it is absolutely necessary that the specimens be thoroughly dehydrated, or a cloudiness is produced of watery vapor, and the slide will be sadly ruined until the cover-glass is removed and a new mount is made.

The glass generally used for holding the object is made of the best white crown glass (Fig. 63), measuring 76 x 26 mm., or 48 x 38 mm., the edges of which are ground. The cover-glasses are thin circles (Fig. 64), cut to dimensions of 12, 15, 18, 21, and 24 mm. Square cover-glasses (Fig. 65) of the same dimensions can also be purchased from dealers, when such shapes are desired. The square forms are not suitable, however, when the manipulator wishes to use the turn-table to form a ring before and after placing on the cover-glass. The thickness of the cover-glasses varies from 0.15 to 0.22 mm. When the immersion objective is used, it becomes desirable to know this thickness.

¹ Damar is also used for mounting sections and by some is preferred to balsam; Lee, in his "Vade-Mecum," states that "damar gives the better definition of delicate detail; balsam has greater clearing action, and affords perhaps more solid mounts" (p. 249).

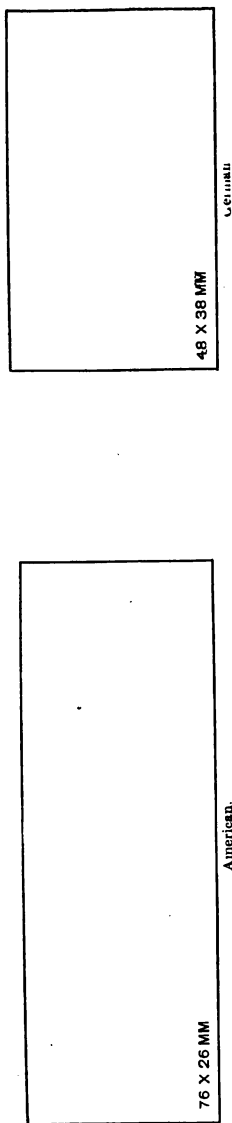


FIG. 63. — Glass Slips.

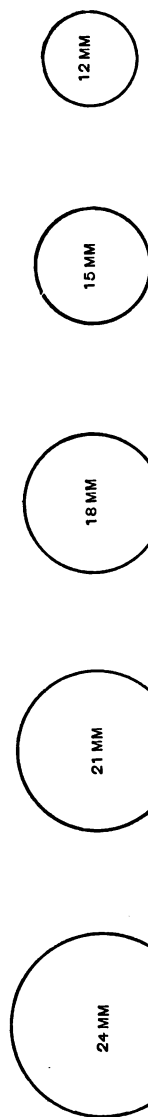


FIG. 64. — Cover-glass Circles.

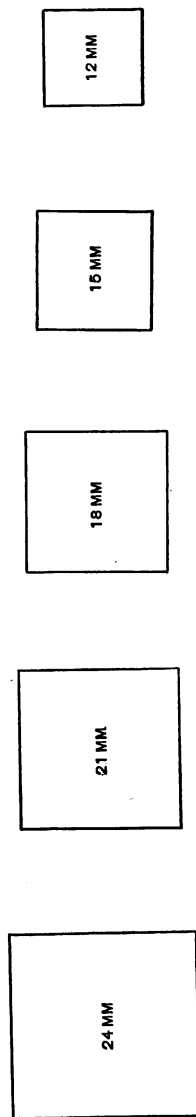


FIG. 65. — Cover-glass Squares.

Of course it is important that these glass slips and covers should be scrupulously clean, free from all films of grease and other extraneous matters, before the sections are fixed; otherwise serious trouble awaits the operator. The cementing ring will fail to attach firmly to the surface, and the mount will present a slovenly appearance. For recipes suitable for cleaning the glasses, the student is referred to the pages at the close of this volume.

The mode of procedure for making the complete mount is as follows: If the sections do not require a previously prepared ring for the glass cover, place the slip on the turn-table and centre accurately. By means of a needle put a drop of Canada balsam on the sections, and the proper size cover-glass is grasped between the forceps and the edge of the glass cover opposite to the forceps is first brought in contact with the slip and then gradually lowered until nearly in contact all around, when the forceps are removed and the circle is permitted to fall gently into place. A spring-clip is now placed on, so that all superfluous balsam is driven out, as well as most, if not all, of the air bubbles. If a few very small air bubbles remain, these will pass out when the balsam begins to thicken, in the course of a few hours.

If the sections are too thick, however, to take the cover-glass without a ring to raise the cover above the slip, the slip should be prepared with a ring some time before the sections are placed in position, in order to give the gum composing it time to thoroughly harden. This primary ring can be as high as desired by repeated applications after each coating has had time to first dry and harden. In making the mount, the edges of the cover-glass should reach only as far as the centre of the ring, and it will then be possible to seal the mount with the last ring.

These gummed rings are made of either Brunswick black, shellac, King's cement, asphalt, or other like solutions, dissolved in xylol, benzole, alcohol, etc. The spinning is accomplished by means of a small camel's-hair brush, held with a steady hand in contact with the glass slip, while the table is rapidly

whirled by the other hand. The brush should be so held that a line drawn from the person through the centre of the table will make nearly a right angle with the line passing through the length of the brush handle. The cell wall should be one-sixth of an inch wide, and should project about half that space beyond the edge of the cover-glass.

After seasoning for two or three days, or longer if required, to well harden the balsam between the cover-glass and the slip, the mount is placed on the turn-table again and, by means of a scalpel, the balsam remaining outside of the cover is carefully scraped off as the table is slowly turned. A cloth, moistened in benzole or xylol, is rubbed over those portions of the glass still containing films of balsam, extra care being taken, however, to avoid displacing the cover-glass and dissolving the hardened balsam under its edge, which cements it to the glass slip. All balsam must be removed and the benzole dried off to prevent the finishing ring from running when it is applied. When this cleaning is *thoroughly* completed, the cover-glass must be permanently sealed with a ring of gum, which will prevent the access of air or displacement of the cover in after handling. This finishing ring is put on in the same manner described for making the cell wall; and the precaution is taken to spin with a cement which has a solvent different to that in which the balsam is dissolved to prevent its running under the edges of the cover and staining the balsam, because it must be remembered that the same solvent will dissolve both. For instance, benzole and xylol are solvents of balsam; a ring, therefore, made of a gum dissolved in either benzole or xylol will run when the balsam comes in contact with it. In this case, then, it is best to make the ring of a cement which is soluble in alcohol, like King's, or one soluble in water. In spinning the final ring, bring the brush in contact with the circle and slip at the same time, so that the ring will lap on each, and thus make a perfect sealing. The slide is now properly labelled and placed aside to dry. If the damar cement is preferred, the steps are the same as those given in the case of balsam.

Glycerin jelly mounting. — The fixing of the sections to the slip and staining are in all respects the same for mounting in glycerin as already described for mounting in balsam. A small lump of the glycerin jelly is transferred to the centre of the cover-glass, and warmed in the water-bath. As soon as the glycerin melts, the slip also warmed is centred on the turntable and the cover-glass put in place with the forceps the usual way (see page 129). Care must be taken that the heating in the water-bath is not greatly prolonged, because there will be danger of introducing many air bubbles in the glycerin. If air bubbles should appear, they must be exploded before the cover-glass is put on, and a close examination must be made for them by means of the hand magnifying-glass. By placing the jelly on the cover-glass instead of the slip, the chances for air bubbles are greatly reduced. With the spring-clip in position on the slide, the surplus glycerin is carefully washed off with water and the surface of the glass thoroughly dried by means of blotters. A ring of the glycerin jelly is now spun around the edges of the cover-glass and the slide is put aside under a bell-glass to season and harden, after which the finishing gum (dissolved in benzole or xylol) is spun over the jelly and the slide is then labelled. The jelly, in each case, must be allowed to congeal before the gum is spun on. The student is referred to the chapter on recipes for a suitable cement for finishing his slide.

Dry mounts. — It is only necessary to use mounting media in those cases where preservation is demanded, but in some instances the specimen may be mounted without the media, particularly when the slide is intended only for temporary use, and there is no desire permanently to preserve the form. Under these conditions the specimen is attached to the slip by means of some transparent cement, — Canada balsam will do very well, — and a *thin* ring of cement is spun around the well-seasoned cell, and the cover-glass is firmly pressed in contact with it, and the slide is then placed aside to dry, after which the finishing ring is applied if desired.

CHAPTER VIII

HOW TO MAKE DRAWINGS OF THE SECTIONS OF TISSUE

BEFORE photography became so widely used — anterior to the introduction of dry plates and the many facilities for rapid and comparatively easy working — drawing was the only method available for readily transferring to paper what was revealed in the microscope. Even now some microscopists of distinguished abilities prefer drawing to photographing the image. It is the opinion of the author that both methods should be used ; drawing in some cases will be found best, while in others photography will give the most satisfaction. Drawing must be entirely free-hand, and, of course, where the results are largely due to the judgment of the observer, the reproduction cannot in all particulars be as accurate as the exact impressions made on the sensitive plate by the photographic methods. But in order to secure the best results with the photo-micrographic apparatus, the sections must be very thin, so that all parts will be in focus when the picture is taken ; otherwise an unpleasant blur will be produced. With some kinds of objects it is impossible to avoid the presence of several planes, and the readjustment of the microscope becomes necessary in order to bring out each part of the object clearly. Such specimens can only be reproduced by the camera lucida. The operator must be constantly raising and lowering the objective while drawing the details ; such manipulation, of course, would be out of the question while using the photo-micrographic camera. The student must, therefore, learn to use both the camera lucida and photo-micrographic outfit.

The necessary apparatus required for drawing the image consists of a compound microscope, a camera lucida, stage and

eyepiece micrometers, liquid India ink, sharp-pointed steel pens, and good lead pencils. Bernhard's drawing-desk, or Bausch & Lomb's modification, may also be added as a convenient aid, though not absolutely necessary.

The camera lucida. — This instrument is attached to the ocular end of the microscope and is used for directing the rays, which emanate from the object, in such a manner as to cause the virtual image to appear projected on the table at the foot of

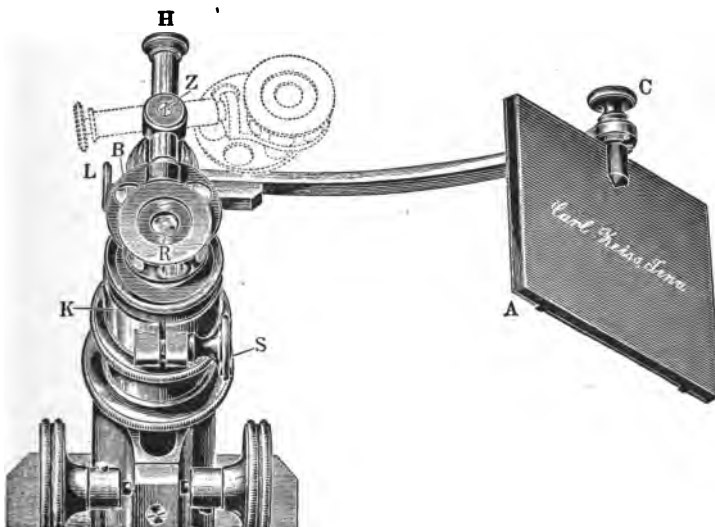


FIG. 66. — Abbe's Drawing Camera Lucida.

the microscope, where it may be drawn with a pencil. The fields of view on the drawing-board, and on the stage of the microscope, are both combined in one eye, and the image on the drawing-board is made visible by double reflection. Two of the best camera lucidas in the market are those made by the Zeiss Optical Company, of Germany, and Bausch & Lomb Optical Company, of Rochester, N.Y. These are shown in the cuts (Figs. 66 and 68). "The drawing-surface is made visible by successive reflections at a large plane mirror and the silvered

surface of a small prism in the eye-point of the eyepiece. The microscopic image is seen directly through an aperture in the silvering of the prism, to which is cemented another prism, both prisms together forming a cube. Thus the pencils of light reached the eye coincidentally from both the microscope and the paper, and the image and pencil are seen without straining the eyes."¹ The rays from the drawing-surface first pass through two tinted glasses of different degrees of color, which serve to equalize the illumination of the field and paper, and they will be found necessary when high powers are used for drawing. The

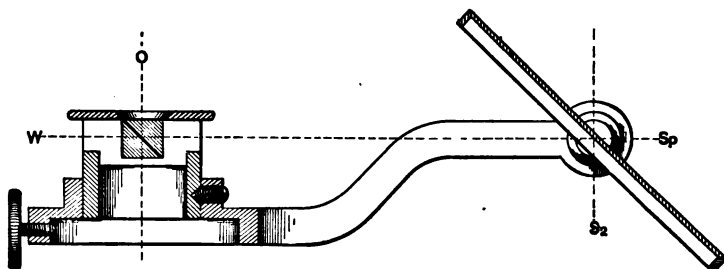


FIG. 67. — Section, Abbe's Camera Lucida.

arm upon which the mirror is poised (Fig. 67) is 10.5 cm. in length, and it is not required to raise the surface of the drawing-board unless in the case of very large drawings. This lengthening of the mirror arm greatly lessens the tendency to distortion. If the drawing is large, it will be necessary to raise or incline the surface containing the paper. The camera lucida illustrated in Fig. 66 shows a material improvement by Abbe, in substituting for the tinted glasses a movable cylindrical cap *R*, in the wall of which is placed a series of tinted glasses, and by turning the cap on its upper edge until a small pin engages in a corresponding small hole on the lower edge of the cylinder, each smoked glass may, in turn, be brought in the path of the rays from the drawing-surface. In the cylinder are five tinted glasses of different strengths, while the sixth hole is left empty.

¹ Zeiss' "Microscopes and Microscopical Accessories," p. 82.

The Bausch Lomb Optical Company's camera lucida (Fig. 68) is made after Abbe's pattern, but differing from the German instrument in the following particulars:—

1. The mirror arm is adjustable so that the distance between the mirror and the prism may be increased or diminished at pleasure.
2. The mirror is extra large, thus increasing the surface of reflection and correspondingly giving a wider range on the drawing-board for drawing the image.
3. A second series of tinted glasses are arranged to modify the light coming from the mirror. With the two series of colored

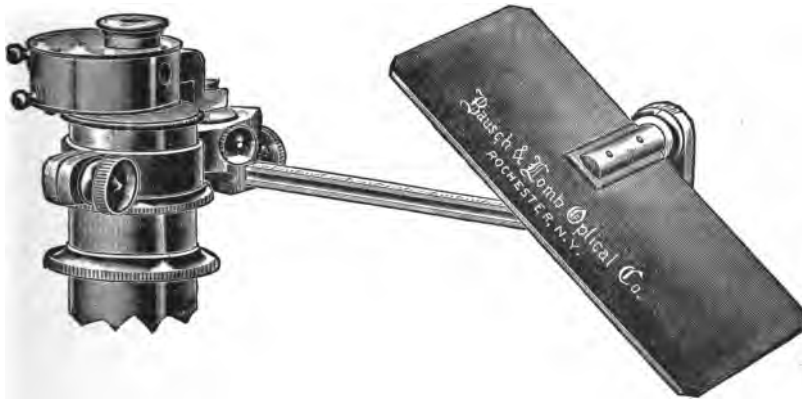


FIG. 68.—Camera Lucida—Bausch & Lomb.

glasses it is possible to secure a clear view of the object with the combination of any objective and ocular.

For diminishing the brightness of the image a disk *B*, as in Bernhard's apparatus (Fig. 69), with four smoked glasses and a vacant space, is interposed between the prism and the eyepiece. In order to pass conveniently from observing the image projected on the drawing-board to observation directly through the ocular, the instrument is so constructed that the prism and the diaphragm may be turned on the pivot *Z* to one side and the microscope can be used as though the camera is not clamped to

the tube. The eye-circle is accurately centred with the aperture in the prism by means of the two screws *H* and *L*. When this is completed, there should be no obstruction to the vision through the ocular field. Provision is also made in the instrument for a spectacle-glass, in order to render the drawing-surface more distinctly visible. This fits in the recess in the top of the cap *R*. It is recommended by the manufacturers that in order "to obtain a fairly large field, drawings free from distortions, a slanting or

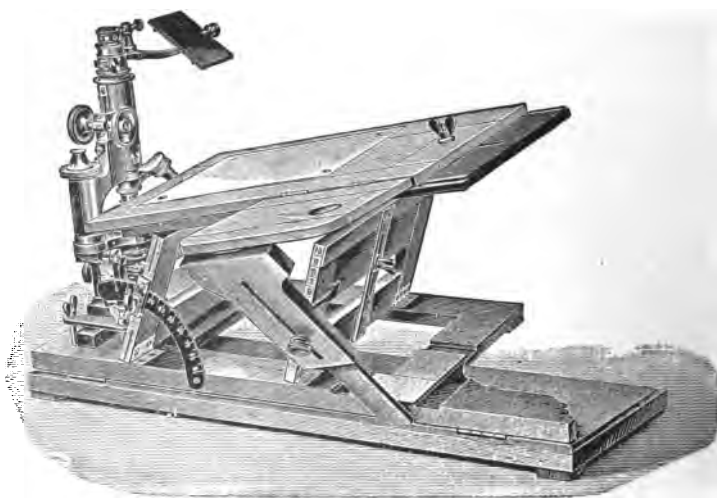


FIG. 69. — Bernhard's Drawing-table.

raised drawing-surface should be used." The drawing-desk devised by Dr. W. Bernhard is most admirable for this purpose. The following points in its construction make it particularly well adapted for its purpose: The drawing-board has an exactly worked brass groove on the upper plate, so that any shifting of the parts is prevented. The height and inclination of the board are controlled by means of a graduated arc. There is an adjustable hand-rest provided, and it is hinged to the drawing-plate. A supporting piece is hinged beneath this hand-rest, which

is capable of extension. The entire apparatus can be inclined from the plane of the table upon which it is resting, by means of hinge connections with a solid base-plate. This is shown in the cut. "The plane of the drawing must be at the normal distance of distinct vision, *i.e.* 250 mm. from the eye of the observer, since in general the drawing should correspond in its dimensions

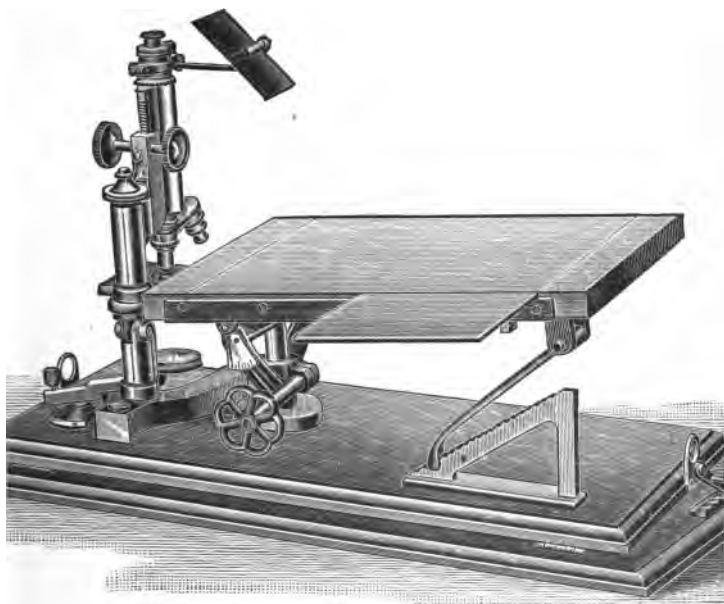


FIG. 70. — B. & L. Drawing-table — Bernhard's Modification.

with the microscopic magnification, and therefore also the drawing-desk must be adjustable in height and inclination to the microscope."¹

In the drawing it is generally customary to mark off only the outlines of the image on the paper, and the details are afterward put in without the aid of the camera lucida. The size of the

¹ *Zeitschr. f. wiss. Mikr.*, Vol. XI, p. 439, 1892, and Vol. XI, p. 298, 1894. Also *Jour. Roy. Mic. Soc.*, p. 782, 1893.

drawing is determined by the aid of a micrometer located in the ocular. Before beginning the drawings the ocular micrometer must be carefully compared with a stage micrometer, in order to determine its exact value, and it will be advisable to construct a table of known standard measures for each of the objectives, and then the stage micrometer may be placed aside and the ocular micrometer used instead. When the paper is put in position on the drawing-board for reproducing the image, and the camera lucida has been adjusted in its place, the measurements should be marked on the margin of the drawing to show the degree of magnification of the image. When the work is finished in ink, this measurement should also be permanently marked on the paper. In work of a high grade, this indication of the magnification is absolutely necessary, and should always be shown on the drawings to make them of scientific value. The simplest way of securing this scale is to place the stage micrometer on the microscope and draw its lines through the camera lucida on the margin of the drawing. Put the object in the place of the stage micrometer and proceed to draw it in its proper place on the paper. Now in order to determine how great the enlargement is, apply to the drawing of the image of the object a millimeter rule and note how many of the spaces on the scale drawn on the paper are covered by this measure of the image, and the magnification will be obtained by dividing this number on the millimeter rule by the known value indicated on the stage micrometer. Thus suppose the drawing of the image covers 3 spaces on the scale made on the paper, and these 3 spaces measure 45 millimeters on our rule, then we will have $45 \div .03 = 1500$ diameters.

The stage micrometer is divided into hundredths of millimeters, and, therefore, the 3 spaces on our drawing would represent .03 mm. on the micrometer. If we are using the micrometer ocular in the work before us, we must proceed as follows: First secure the ratio existing between the stage and the ocular micrometers, and then continue the calculations given above. To obtain this ratio, place the two micrometers in their proper positions on the microscope and note how many divisions on the

stage micrometer are covered by those on the ocular micrometer, and then our rule will be : Divide the number given by the stage micrometer by the number given by the ocular micrometer. To illustrate, suppose 5 divisions of the stage scale correspond to 15 divisions on the ocular scale, then the ratio will be : —

$$\frac{.05}{.15} = .0033 + \text{mm.}$$

Now having obtained this ratio for the objective and ocular in use (and this must be done for each combination of the objective and ocular), the size of the object can be found by dividing the number of divisions on the ocular micrometer, covered by the image of the object, by the ratio given for the two micrometers. Thus, if the image covers 8 divisions on the ocular scale, the magnification will be : —

$$\frac{.08}{.0033} = .024 + \text{mm.}, \text{ or } 24 \mu.$$

In drawing the object thus indicated, great advantage accrues to the student in making him careful about details and accurate in regard to results. He will find that a poor mount cannot be well drawn, and he will, therefore, be careful from the start to make all his work the very best possible. In doing this drawing, the following items may be kept well in mind : —

1. Use hard pencils, with fine, sharp points.
2. The drawing can be best made on Bristol board, or similar smooth-surfaced paper.
3. The light which comes through the tube of the microscope from the object, and also those rays which come from the image and are reflected by the mirror of the camera lucida to the eye of the observer, should be of nearly the same intensity, in order to bring out clearly both the point of the pencil and the image. There are several ways of regulating the intensity of the light at each point. If the stage is too bright, the Abbe sub-stage illuminator will readily correct the trouble. If the microscope is not provided with the Abbe illuminator, then the diaphragm must be used with a smaller opening, or a screen must be placed between the stage and the source of light. To know which

field is the brightest, the student must watch the image of the pencil and of the object, and if the latter is the most distinct, then he will at once know that the stage of the microscope is too bright; if the point of the pencil is the most distinct, then the surface of the paper has too much light on it. The character of light coming from the surface of the paper can be controlled by the camera lucida, if Zeiss's pattern is used, as has already been indicated. This, however, may be accomplished also by placing a screen of ground glass between the source of light and the drawing-board. These mechanical devices, however, for controlling the quantity of light can be multiplied by the ingenuity of the student.

4. Fasten the paper securely to the drawing-board, so that it will not be displaced during the operations.

5. Follow faithfully the lines of the image, taking care not to make heavy markings with the pencil. Make all cell walls, of an appreciable thickness, with double lines, and the solid portions of the sections should be well shaded.

6. Note on all drawings not only the magnification, but also as well the number or letter of the objective and ocular. This is not often done on drawings, but it is very desirable when work of a high grade is performed, in order that satisfactory comparisons may be made at any time.

7. If the outlines of the image are made with a low power, and the details are put in with a higher power, this fact must be clearly indicated on the drawing.

8. The eye must be held close to the microscope, and look perpendicularly down, so as not to distort the image and produce an incorrect drawing.

9. The height of the drawing-desk must be lowered or elevated until its surface is brought into the distinct visual distance of the observer. This height can be determined by the distinctness of the inclined surface of the desk. Draw the measurements of a stage micrometer on the paper, and if the lines of the divisions stand at unequal distances, separating more and more, as you approach the top of the desk, the surface needs inclining.

CHAPTER IX

PHOTO-MICROGRAPHS. THE APPARATUS

THE remarkable strides made in the science of photography within the past few years have rendered it possible to reproduce with great exactness the image of the object, and with such a degree of facility as to place the results within the reach of any microscopist who possesses a general knowledge of chemical laws, and who will exercise the ordinary degree of care in the use of delicate apparatus and chemicals.

Before entering upon a discussion of the methods used for making the negatives and positives, it may be best to give a detailed account of the apparatus and chemicals required for a satisfactory prosecution of the work. In the descriptions which follow, it is assumed that the student is a novice in the science of photography, and therefore the subject is treated fully in order to make the methods clearer to the minds of the beginners.

As in the case of the microscope and its accessories, so is it true in reference to the photographic outfit: the best apparatus made by the most skilful workmen will be the cheapest in the end. A lens made to sell and offered below what is a reasonable price for a first-rate glass will soon be found inadequate to meet the demands, and only inferior pictures will be the results.

The camera. — The following sizes of view cameras will be found very convenient and useful in the laboratory: 4×5 and $6\frac{1}{2} \times 8\frac{1}{2}$. The different sizes of kits should be purchased with the cameras, so that the several sizes of negatives may be made without requiring the purchase of other cameras. It will be possible to do all needed work with the cameras above-mentioned, but in a well-equipped laboratory it will be found desirable for convenience to add also a photo-micrographic camera,

either Bausch & Lomb Optical Company's or Zeiss's pattern. These last are very expensive, but the laboratory that contains one of these outfits for photo-micrographic work is prepared to accomplish high-grade results with the least degree of inconvenience.

As previously stated, two sizes of cameras will be sufficient for most of the photographing required in the laboratory. The 4×5 camera may be of the Kodak style or the Premo pattern; but this apparatus should have an extra length of bellows, so



FIG. 71. — Folding Kodak Camera.

that the instrument can be used for many purposes requiring a long bellows. There are several good hand-cameras on the market, like the Kodak and Premo, any one of which may be used instead of the two mentioned, provided the essentials found in these two excellent instruments are secured.

In the following description of the hand-camera the cartridge kodak is taken as the pattern of the type of these convenient instruments, not that it is considered by the author to be the best, but because it has some additions which are not found in others.

The kodak may be used as a hand-camera (Fig. 71), or it may be mounted on a tripod when exact focussing is desired. The meaning of the term "hand-camera" is in reference to those instruments which are so constructed that they can be held in the hands when used in taking "snap-shots," or pictures of objects which are moving, or of bright objects when the exposures are made as short as $\frac{1}{25}$ of a second, or shorter. It is wise to purchase with the camera a tripod, in order that delicate focussing may be done at any time desired. This is a support for the camera, so constructed that it may be folded up



FIG. 72. — Camera Tripod.



FIG. 73. — Carrying-case for Tripod.

when on a trip, and stored away in the carrying-case (Fig. 73). It will always pay to place the camera on the tripod and carefully focus the picture on the ground glass, if the object to be photographed will permit of such treatment, because it is almost impossible to secure sharp photographs by holding the camera in the hands and snapping at objects, the distance being only guessed at. In some instances, however, the tripod cannot be used, and then the value of the instrument as a hand-camera becomes quite evident.

There is always attached to these cameras a "view finder," which is a small lens reproducing in miniature, under the eye of the photographer, a picture of the object desired on the sensitive plate. This view finder enables the photographer to watch the moving object, and just as it moves across the field of the instrument he can snap the shutter in front of the lens and take the picture.

At the rear of the camera is located the ground-glass plate, on which to focus the picture of the object when the tripod is used and time exposures are made. The bellows allows the front and rear of the instrument to be adjusted so that focussing may be accomplished, and the camera may be folded up and placed in the carrying-case during transportation and to exclude dust when the instrument is not in use. The hand-cameras now made are constructed to fold within themselves into a compact form, and, being covered with neat leather, they do not require a case.



FIG. 74. — Roll-holders.

In connection with these hand-cameras roll-holders are used (Fig. 74), in which a roll of thin celluloid highly sensitized is placed, and so mounted in the holder as to permit of being brought by the mechanism, a portion at a time, before the lens to receive the picture. Each roll of film contains six or twelve 4×5 inch surfaces, so that as many as six or twelve pictures may be impressed on the sensitive surface of each roll before reloading the holder becomes necessary. These cartridges, or rolls, are so constructed that the loading of the holder may be done anywhere and in the brightest light. The film is put up in light-tight rolls, as shown in the illustration (Fig. 75). Extending

the full length of the spool and several inches beyond each end of the sensitive film are strips of black paper. This paper is also continuous back of the entire length of the film. When the roll of film is inserted in the holder, the end of the black paper is attached to the extra roll and the holder is closed, so that all white light is excluded. The key is now turned until the sensitive film covers the space behind the lens, when the exposure is made for the first picture. At proper intervals on the back of the black paper, and behind the film, are marked in white the figures 1, 2, 3, 4, etc., representing the number of exposures possible on the sensitive surface of the film. These numbers are visible through a small opening in the back of the holder, which is covered with red celluloid. When the last exposure is made, a few more turns are given to the key, so that the extra length of black paper may be also wrapped on the roller and cover the sensitive surface



FIG. 75. — Roll of Film.

of the celluloid, and then the roll is taken out and a fresh roll is inserted in its place in the holder. All of this may be done in white light, if care is exercised to manipulate the black paper properly at the end of the sensitive film. The best hand-cameras in the market are constructed for use with the roll-holder, and they also have attached facilities for the use of sensitive glass plates and cut films.

The larger camera, of $6\frac{1}{2} \times 8\frac{1}{2}$ inches, may be any make, provided it is manufactured out of the best materials. There are a number of good instruments on the market which will subserve all the purposes demanded in the laboratory, and they must possess the following important properties:—

1. It must have a double rising front for lowering or elevating the lens without disturbing the position of the tripod. This gives wide range of adjustment.

2. It must have a double swing back, which will insure a

clear, sharp focus on the object ; it makes no difference how the object may be placed in front of the camera.

3. There must be a rack and pinion adjustment, so that the ground glass will remain stationary while the focussing is made on the glass.

4. The ground-glass frame should be hinged to the camera, so that it will not be broken during the time of exposure by some one stepping on it if laid on the floor or ground.

The Lens. — Two sizes of the photographic lens will be found sufficient for the usual work in the laboratory, — 4×5 and $6\frac{1}{2} \times 8\frac{1}{2}$. A wide-angle lens will also be found very useful in certain kinds of work, and it will be well also to purchase one of these for the laboratory. The following lenses are considered to be among the best now offered in the market : —

Alvan G. Clark's Lens.

Beck's Autograph.

Dallmeyer's Lens.

Darlot's Lens.

Francais's Extra Rapid Rectilinear.

Goerz's Double Anastigmat.

Gundlach's Lens.

Morrison's Lens.

Queen's Pantagraph.

Ross's Lens.

Steinheil's Aplanatic Lens.

Suter's Aplanatic.

Voiglander's Lens.

Zeiss's Anastigmat.

The above list may be classified as follows, according to merit : —

- | | |
|--|--|
| 1. Zeiss's Anastigmats. | |
| 2. Dallmeyer's Lens. | |
| 3. { Beck's Autograph. | 4. { |
| Goerz's Double Anastigmat. | |
| Suter's Aplanatic. | |
| 5. The others mentioned in the list may be placed under this head. | Alvan G. Clark's Lens.
Gundlach's Lens.
Voiglander's Lens.
Steinheil's Aplanatic. |

In the selection of a photographic lens, the following requisites must be carefully noted : —

1. Achromatism, of course, is of first consideration, and the lens must stand the test well, because a slight deviation from achromatism will produce an indistinct image.

2. Distortion of the image on the outer limits must be reduced to the least degree possible.

3. Rapidity in action and rectilinearity in results are absolutely required in all first-class lenses. The rapidity of a lens depends upon the size of the stops in the diaphragm. The larger the opening, more light can enter and quicker will be the action on the sensitive plate. The size of the diaphragm is controlled by the focal length of the lens; that is to say, if aperture $f\ 16$ be used, it signifies that the size of the beam of light which enters the lens is one-sixteenth of the focal length. This property of the objective has been carefully worked out by the Photographic Society of Great Britain, as follows:—

Aperture: $f\ 4$ — $f\ 5.6$ — $f\ 8$ — $f\ 11.3$ — $f\ 16$ — $f\ 22.6$ — $f\ 32$ — $f\ 45.3$ — $f\ 64$.

Relative exposure ratio: 1—2—4—8—16—32—64—128—256.

By the adoption of this series of apertures one lens can be compared with another. In the use of the stops it must be remembered that each requires twice the exposure of the one preceding to produce the same results on the sensitive plate. The special advantages in their use, however, consist in reducing to a minimum the blurring of the image on the outer margins, and in producing sharpness in detail which is especially pleasing in the picture. The small stops also increase the depths of definition.

4. The angle of view should not be less than 50° for the rectilinear type, while the wide-angle series should cover an angle of not less than 85° .

5. Depth of focus depends on the angle of view, working aperture, and focal length. As these increase, the depth of the focus decreases, and *vice versa*. Depth of focus is due to the converging rays striking the plate at a more acute angle.

6. Flatness of image over the entire surface of the ground-glass plate by extending the oblique pencils of light.

7. Depth of definition to give a sharp, clearly defined image on any portion of the plate, by reducing spherical aberration. When the focus of the lens is shortened, the greater will be the depth of definition. Thus it is that in the case of a short focus lens there will be projected on the sensitive plate a greater

number of well-defined objects in the foreground than will be secured in the use of a lens of longer focus. This is a valuable property in landscape work, where distance comes in as an im-

portant factor.

The following additional apparatus will be required for making the negatives and positives :

Focussing cloth and focussing glass.

Double plate-holders.

Diaphragm shutter.—

One of the best of these shutters is that made by the Bausch & Lomb Optical Company. The illustration of this instrument is given in Fig. 76. In speaking of this shutter the manufacturers have the following to say in regard to its merits, which the author after several years' use of the instrument can indorse :

“ The construction of the iris diaphragm shut-

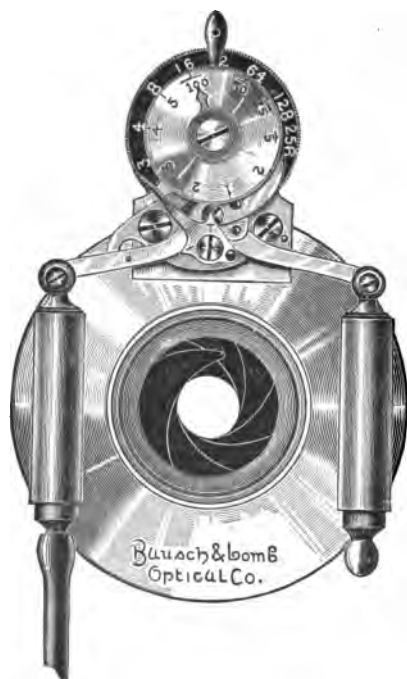


FIG. 76. — Diaphragm Shutter.

ter is based on a careful consideration of the optical principles involved, and it is without question the most scientifically correct shutter yet produced. It can be easily proven, both theoretically and by actual experience, that as the size of the stop is decreased, the lens has not only greater covering power, but the illumination (exposure) becomes more uniform. Furthermore, it is well known that exposures with large openings give greater contrast between the high lights and shadows, while with small stops and long exposures greater detail is obtained in the shadows.

For this reason a shutter constructed on the iris diaphragm principle, opening in the centre with a minute aperture, and gradually enlarging to the full opening desired, then gradually reducing its opening to the closing point, gives the high lights full value and sharpness, depth of focus and detail in the shadows approaching the results obtained with small stops, so that in actual use the diaphragm shutter gives great covering power, more equal illumination, and greater depth of focus than is possible with any other shutter. The following are some of its advantages:—

“It is placed between the systems of the lens, at the diaphragm point, thus acting as stop and shutter at once. No extra stops are needed. It gives absolute uniform illumination over the entire plate. It gives brilliant high lights, and at the same time definitions in the shadows. It gives automatically any exposure from one-hundredth of a second to three seconds, and time exposures for any duration. It has setting device to give any size opening from pin-hole to the largest stop. It is operated either with pneumatic bulb or finger release. It cannot open, or expose the plate, while being set. It does not jar the camera, even when working at the highest speed. It is especially adapted for hand-camera lenses.”

Ruby lamp for the dark room.—This lamp may be either in the form of oil (Fig. 77), gas, or electric lamp. White light must not be permitted to escape from the lamp during the work with the sensitive plate in the dark room in developing the picture and fixing it on the surface of the glass, or film, so that exposure to white



FIG. 77.—Carbutt's Múltum in Parvo Ruby Lamp.

light will not destroy it. The lamp must be made with a combination of ruby and orange glass, or ruby glass covered with orange tissue-paper. The light sifting through such a protection will not affect ordinary sixteen-sensitometer plates, and it is safe for orthochromatic plates and films, provided they are not exposed to it longer than a few moments at a time.

Ray filter or color screen. — In photographing microscopical objects which have been colored with anilin or other dyes, it becomes necessary to interpose between the sensitive plate and

the object a color screen, which will serve to increase or decrease the intensity of certain colors of the spectrum. The ray filter (Fig. 78) is a metal ring or cylinder, with each end closed by a glass disk. In this cell thus formed by the disks and cylinder is placed the



FIG. 78. — Ray Filter.

colored solution for sifting the light before it reaches the sensitive plate. This ray filter is made to place on the hood of the photographic lens like a cap. The solution is bichromate of potash. The effect of this sifting of the colors of light before they reach the sensitive plate is to give a picture which produces true color values, and renders correct gradation of shading and sharpness in details throughout the entire photograph. The ray filter is particularly valuable for making photo-micrographs.

Developing trays of various sizes. — A number of these indispensable vessels should be in the dark room for the various purposes for which they are made. They are manufactured out

of glass, rubber, and other material not affected by the chemicals used in the developing of photographic plates.



FIG. 79. — Developing Tray.

Graduates for measuring the chemicals.

— The following sizes will be found to be useful in the laboratory and in the dark room: 4 ounces, 32 ounces. (Fig. 80.)

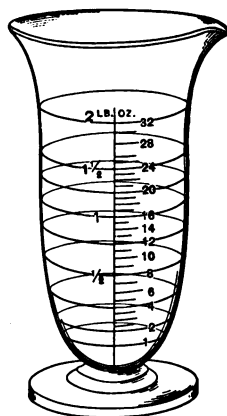


FIG. 80. — Graduate.

Fixing-baths made of hard rubber. — The Gennert pattern is excellent for several reasons. They have grooves extending from the top to the bottom of the vessel (Fig. 81), in which the plates slide and stand in a vertical position, thus preventing injury from the precipitates which form in the solutions. A top covers the vessel, so that both dust and light are excluded during the fixing of the plates in the hyposulphite solution. There are two sizes made, one for plates $3\frac{1}{4} \times 4\frac{1}{4}$, $4\frac{1}{4} \times 6\frac{1}{2}$, and $6\frac{1}{2} \times 8\frac{1}{2}$, and one for plates 4×5 , 5×7 , 5×8 , and 8×10 .



FIG. 81. — Fixing-bath.

Negative washing-bath. — The vessels made of glass are more durable and are easily kept clean. These baths (Fig. 82) are used for eliminating the hyposulphite of soda from the negatives after they have been fixed in the dark room, because if the hyposulphite of soda is left in the film of the negative, the picture will soon be destroyed by the chemical action of the soda on the gelatin film. The plates are washed by running water

into the bottom of the bath, where the plates are resting on their edges; and as the water runs out of an orifice at the top of the vessel, it dissolves out the hyposulphite of soda and leaves the negative free from that destroying agent.

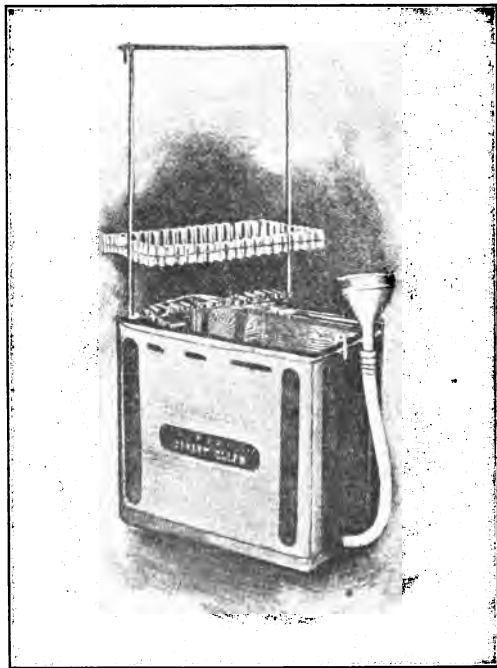


FIG. 82. — Negative Washing-bath.

Light-tight boxes of various sizes for holding the unexposed plates and keeping them from white light.

Printing-frames (Fig. 83), to agree with the size of the plates used in the camera for photographing objects in the laboratory or in the field. The sizes usually found convenient are $3\frac{1}{4} \times 4\frac{1}{4}$, 4×5 , 5×7 , 5×8 , and $6\frac{1}{2} \times 8\frac{1}{2}$. These frames should have a clear glass plate fitted to each, so that celluloid films from the hand-cameras may be also used in them.

Negative drying-racks. — The racks (Fig. 84) are for the purpose of drying the negatives after they have been thoroughly washed and swabbed with absorbent cotton to clear off the particles adhering to the surface of the film.

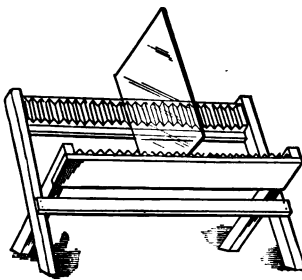


FIG. 84. — Negative Drying-rack.

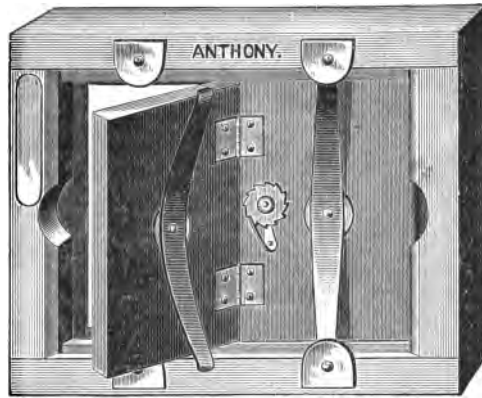


FIG. 83. — Printing-frame.

Retouching-frame. — Even under the most careful manipulation of the photographer, it sometimes becomes necessary to touch the negative here and there with a pencil or brush to smooth over blemishes. The retouching-frame (Fig. 85) is adapted to this work. The negative is placed on the ground-glass plate *A*, and the slide *E* is adjusted so as to

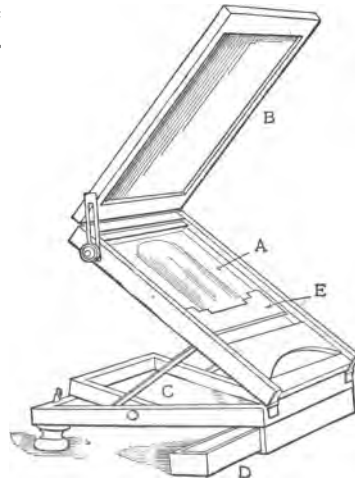


FIG. 85. — Retouching-frame.

hold the plate in position, and the mirror *C* is turned so that the light will reflect up through the negative in order that the

operator may locate the spots to be worked on ; the part *B* is to shade the operator's eyes from the light, and the drawer *D* is intended to hold the pencils and brushes when not in use.

Camel's-hair brush, two inches wide, for dusting the plates before they are put in the holders for the camera. The necessity for this dusting of the plates is to prevent the formation of pin-holes in the negative, caused by the dust particles covering those portions of the plate and thus preventing the full action of the light on the sensitive surface.

An enlarging-easel. — This is an important addition to the laboratory outfit if any enlarging is done, that is, if the operator desires to make a picture larger than can be secured with any one of the cameras belonging to the laboratory. A skilful carpenter can make this easel ; it is only necessary to have a vertical board on which the sensitive paper may be stretched, and the board, fastened to upright supports resting on rollers, so that the easel may be readily moved back and forth to get the desired enlargement.

Exposure meters. — It is a difficult matter, especially with the amateurs in photography, to know just how long the sensitive plate should be exposed to the action of the light in order to secure the best results. To obviate this trouble in great measure, there are a number of exposure meters, or scales, and other devices on the market, some of which are excellent, while others are indifferent. Two of the best of these meters are given below. The author has experimented with them and has obtained satisfactory results.

Wager's exposure meter. — This is an aluminum plate, on which slides two ivory verniers. Printed on the plate and the verniers are values for dates, hours of the day, character of the sky and the objects, diaphragm stops, name and speed of plates and films, and time of exposure. The correct exposure of plate is obtained by knowing the speed of the plate, the hour in the day, the season of the year, and the size of the diaphragm. By moving the ivory slides to the correct positions on the aluminum plate, the time of exposure in seconds and in minutes is read off at once.

Watkins' exposure meter.—This instrument has also the factors mentioned as belonging to the meter above, but the correct time of exposure is obtained by exposing to the action of the light a narrow strip of sensitive paper, while seconds are counted by the vibration of a pendulum attached to the instrument. The movement of the verniers on this meter will give the time of exposure for the plate.

The necessary chemicals demanded in the photographic manipulations will be considered when the formulæ are given in describing the methods for making the negatives and positives. But it must be borne in mind that the purest chemicals should be purchased and used in all photographic operations. Inferior and unsatisfactory results will meet the student unless he observes rigidly this rule.

CHAPTER X

MAKING THE PHOTO-MICROGRAPHS (CONTINUED)

Arranging and adjusting the apparatus.— Some place in the laboratory must be found where there is the least degree of vibration, caused by passing to and fro in the building and from outside influences, and there the table containing the apparatus for focussing and exposing the sensitive plate must be located. If the building has a well lighted and ventilated basement, with a cemented floor, no better place can be secured for performing all the work in photo-micrography. It must be remembered, however, that wherever the apparatus is located, decided vibrations given to the table will sadly spoil all results by producing a blurred picture. All parts of the apparatus used for holding the slide, the camera, and focussing appliances, and the condensers for concentrating and controlling the distribution of the light, should all be fastened to the same rigid base, so that if slight vibrations occur in the room, the entire outfit will move as a whole, and there will be less tendency to blurring the negative.

Since the temperature of the atmosphere influences, to a very large degree, the results secured in photographing, it will be of great advantage to have the room under perfect control, so that the temperature may be lowered and raised at pleasure. It has been found, from experience, that the best negatives are made when the temperature of the chemicals range from 15° to 21° C. If these solutions used in developing are not kept within this range, the negatives will be unsatisfactory. Cold retards the results, while heat accelerates and causes flatness. In summer, therefore, ice should be kept around the vessels containing the developing solutions until the temperature is lowered to the required degree, and the dark room must also be regulated, as far as possible, to reach these same results.

Although there are several excellent brands of dry plates on the market, all giving good results in the hands of the careful manipulator, still, it will be wise to study the qualities of the plates made by one manufacturer and use them in most, if not all, experiments conducted in the laboratory. In determining this question, careful notes should be taken of each exposure, and also the action of the developing fluids on the plates. By this method all of the capabilities of the plates will be fully understood. The experience of the author has convinced him that, for general scientific work, Carbutt's plates are superior to most of the brands sold in America. This conclusion, however, may be due to the fact that he is more familiar with these plates than with the others; but, as has been already stated, the same amount of experience with the others may yield as good results. Carbutt's plates give brilliant negatives, and they allow considerable latitude in exposure and development. The following represent the brands he manufactures which are suitable for the experiments in the laboratory:—

“Eclipse, sensitometer 23 and 27.— Films and plates are extremely sensitive, and specially intended for quick studio exposures, concealed and detective cameras, instantaneous views, and magnesium flash-light photography.

“B plates, sensitometer 16 to 20.— For landscape views and general photography.

“Orthochromatic plates, sensitometer 23 to 27.— Give correct color values when exposed through ray filters or color screens. The best plates for landscapes, interiors, and photo-micrography.

“Polychromatic plates.— Color sensitive to entire spectrum.

“Non-halation plates with orthochromatic quality.— These plates are suitable for subjects that transmit much light, like windows and bright polished metal surfaces.

“Cut films are also manufactured by Carbutt, containing the properties described in the above glass plates.

“Process plates make good photo-micrographs, producing clear, sharp detail and abundant contrast.

"Lantern plates. — Giving results of great brilliancy and fine color. For lantern slides and window transparencies. The sensitive film is mounted either on glass plates or celluloid.

"The sensitometer number on each box of plates indicates their sensitiveness; the higher the number, the greater the rapidity of the plate. For portraiture on the special and eclipse plates under the skylight, if an eclipse sensitometer 27 required 2 seconds, a special sensitometer 27 would require $3\frac{1}{2}$ seconds, and a special sensitometer 24, $4\frac{2}{3}$ seconds." (Carbutt.)

The following firms also make standard plates, which are highly commended by both professional and amateur workers, and, as was stated on a previous page of this book, the student should find the brand of plate which suits his experiment, become familiar with the qualities of that brand, and do most if not all of his work with it. Trying first one plate after another is not conducive to good work and satisfactory results.

The M. A. Seed Dry Plate Company make two brands of orthochromatic plates, one of which is very rapid.

The G. Cramer Dry Plate Company have slow, medium, and instantaneous isochromatic plates, which are highly esteemed by some microscopists for photo-micrographic work.

The Stanley, Hammer, New York, and Eastman plates are some other brands which receive commendation from photographers, but it is not the fortune of the author to be familiar with these plates, and they are simply mentioned because commended by workers of known ability in microscopical science.

The celluloid films put up in daylight rolls by the Kodak Company have already been mentioned on a previous page, and the student is referred to what was said concerning these films in that portion of this book.

The orthochromatic plates are the best for all photo-micrographic work, because they give color values, so important in photographing stained sections. Without these plates it would be impossible to secure clear, sharp pictures of the colored sections, because the ordinary dry plates have not the proper tone values, some of the colors appearing too dense, while others

are too light. The plate is orthochromatized by immersing it in a solution of a dye belonging to the eosin group, in accordance with the theory of Professor Draper, who discovered that only those rays which are absorbed by the film on the plate produce chemical changes in the sensitive surface. In using these orthochromatic plates it is necessary to interpose between the lens and the surface of the plate a color screen in order to intercept and equalize the chemical action of the different colored rays which are emitted from the section. The color of the screen is generally yellow, although it is claimed by some experimenters that a tinge of red, blue, or green is sometimes preferred. In the use of these plates two facts must be borne in mind: The exposure must be prolonged beyond that period given to the ordinary plates, and the developing-tray must be covered while developing, so that the plates will not be fogged by the light sifting through the ruby glass. In order to have the dark-room window perfectly safe, it will be well to cover the ruby glass with two thicknesses of yellow paper or cloth specially prepared for this purpose.

Photo-micrographic apparatus.— This apparatus is generally united into one series and consists of the following pieces:—

1. A compound microscope.
2. A camera with a long bellows.
3. A massive cast-iron sole-plate.
4. Optical bench upon which the accessories and auxiliaries are placed in a line one behind the other; the accessories consisting of—
 5. Absorbing cell to extract heat rays.
 6. Condensers for bringing the light to focus.
 7. Iris diaphragm.
 8. Ray filter containing the coloring solution for photographing colored objects with orthochromatic plates.
 9. The light, either oil, ordinary gas, acetylene, electric, or sunlight.

Several firms have given to the microscopist some excellent combinations of the above apparatus, so that photo-micrographic

work is now accomplished with the least degree of inconvenience. It simply becomes a question of how much money the laboratory is able to invest in this important adjunct to the outfit.

One of the most useful simple photo-micrographic outfits on the market is the one illustrated in Figs. 86 and 87. It is made

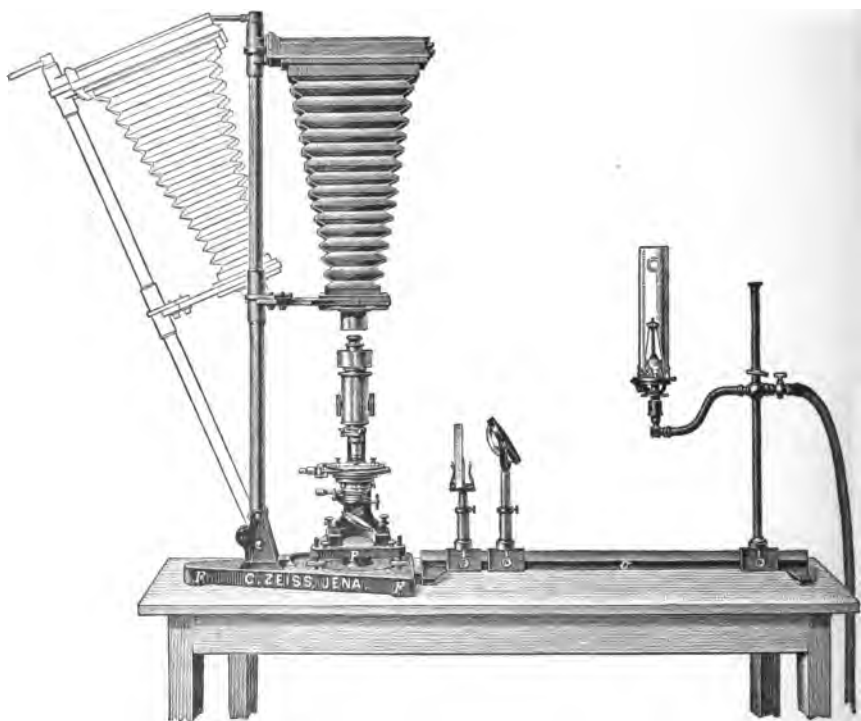


FIG. 86. — Combined Horizontal and Vertical Photo-micrographic Camera.
(Zeiss.)

by the Zeiss Optical Company and is constructed for working in either a vertical or horizontal position. The illustrations give a very clear conception of this instrument. Any first-class compound microscope can be adapted to this outfit. The light is obtained from the gas-mains with the use of the Welsbach

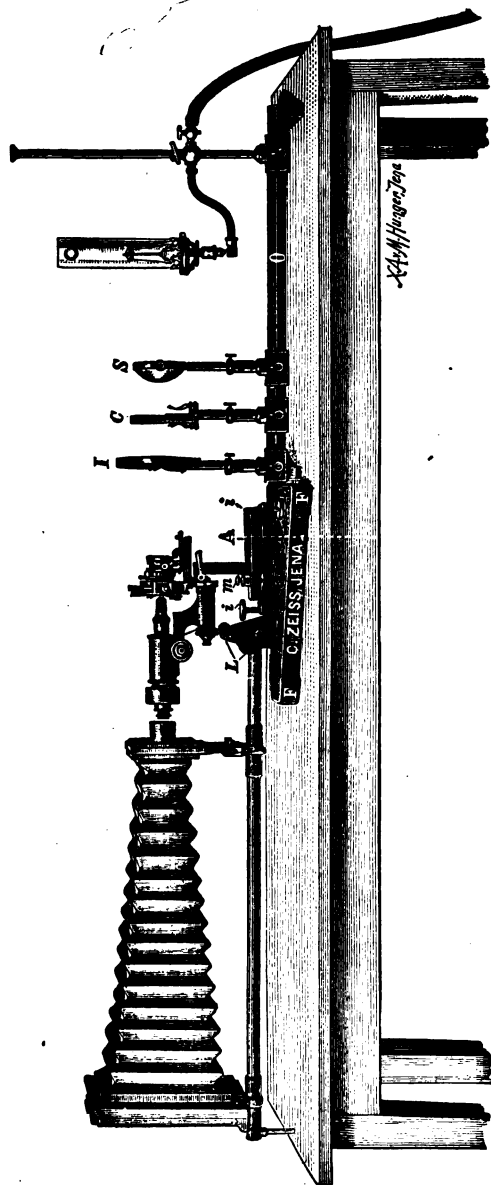


FIG. 87.—Combined Horizontal and Vertical Photo-micrographic Camera. (Zeiss.)

mantle. In using this instrument, or any other of the same kind, care must be taken to see that the connections between the camera and the microscope are securely made, so that no white light can possibly enter the apparatus except that which comes through the objective at the proper time.

When the income of the laboratory will permit of a larger appropriation to this department than is required for the purchase of the last-described photo-micrographic outfit, the author would advise the addition of the larger combination like that shown in the illustration (Fig. 88), or the equally valuable one made by the Bausch & Lomb Optical Company. The one given in the illustration is made by the Zeiss Optical Company of Germany, and is complicated, but very complete in the efficient working of all its parts. The laboratory having such an outfit in its equipment is indeed fortunate, for there seems to be nothing wanting to enable the operator to produce photo-micrographs of the highest degree of perfection.

The essential parts of the outfit are:—

1. An adjustable sole-plate, on which rests the microscope. Underneath this is an elevating-gear for raising or lowering the sole-plate when microscope-stands of varying heights are used.

2. A horizontal prismatic steel rail, on which is arranged axially the parts of the apparatus for controlling the action of the light. This rail is called the "optical bench." See *sr*, page 163.

3. On this optical bench are the following parts: A double lens *S*, for the object of rendering the rays of light parallel; a single front lens *S'*, which serves to bring the rays to a focus; a water chamber *A*, containing water interposed between the two lenses in order to absorb the heat rays; a ray filter *C*, for use with orthochromatic plates and for color values; iris diaphragm *I*, containing spring-clips for holding ground glass to use in centring the light, for colored screen, and such other purposes.

The optical bench and the sole-plate with the microscope, *Mk*, are located on a firm iron table with a wooden top, which

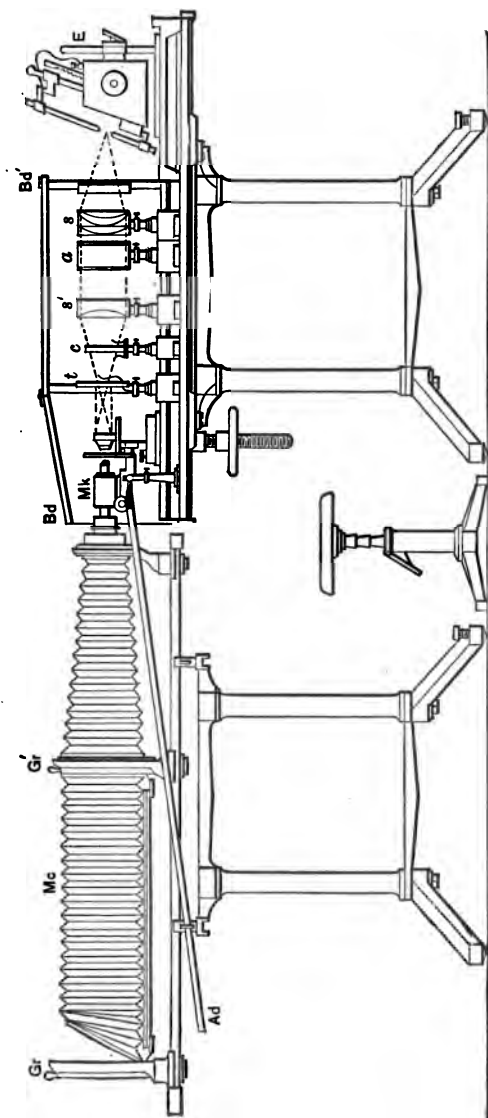


FIG. 88. — Zeiss' Large Photo-micrographic Apparatus, with Colt's Electric Lamp.

gives great solidity and prevents vibration when properly located. On one end of this table is also placed the lamp, *El*. The apparatus from the lamp to the microscope is covered with a board, *Bd-Bd'*, and on each edge of this board is suspended a dark cloth for the purpose of excluding all side light after the apparatus is adjusted and the plate is in position ready to be exposed.

On the second table *TT'* are the camera and the adjusting rod *Ad*, which is used to make sharp focussing when the operator is sitting at the ground glass *Gr*. The camera bellows is 150 cm. long and is made in two parts, so that the camera may be shortened one-half when desired, and a second glass plate at *Gr'* is used for focussing the image of the object, when a less magnification is required.

4. Lantern-slide carrier, with micrometer movement.

5. Movable dark slide for determining the sensitiveness of the plate with the different powers of objectives. The plate is moved through the slide in such a way as to expose only a small strip at a time, so that it is necessary to use only one plate to determine the ratios.

The Bausch & Lomb Optical Company's photo-micrographical outfit is built somewhat on the same general plan as that just described; there are some advantages in one that are not to be found in the other; for instance, in a recent improvement in the Bausch & Lomb apparatus the table carrying the optical bench and microscope is made with only one leg and tripod base, and the top is fixed so that it will revolve horizontally. This enables the operator to turn the table-top to one side and focus the microscope before projecting the image on the ground glass. This is a considerable advantage over the Zeiss pattern, because it enables the operator to avoid the uncomfortable position required in adjusting the microscope.

CHAPTER XI

PHOTO-MICROGRAPHS (CONTINUED) — SUNLIGHT AND ELECTRIC LIGHT, OXY-HYDROGEN, ACETYLENE, CITY GAS, AND OIL LAMPS, AND HOW TO USE THEM

WITH the great improvements developed in electricity within the past few years, the microscopist has but little room for complaint in regard to the lighting of the apparatus for photographing his mounts. In those places, therefore, where electricity can be secured no other kind of illumination should be thought of in photo-micrography. But it is not in the power of every student to use this most excellent light, and he is forced to look in other directions for illumination. Sunlight is, of course, preferable, but there are two difficulties in the way of its use: (1) Its intensity is a very uncertain quantity in doing delicate work, and it is exceedingly difficult to determine the exact time of exposure in each instance while the sun is gradually climbing the zenith, and the rays are becoming constantly more and more powerful in their effects on the sensitive plate. (2) The sunlight is not always available, because of the occurrence of cloudy weather.

When it is possible to use the sunlight, and the operator prefers to do so, there must be attached to the apparatus a heliostat and porte-lumière, or similar contrivance, for controlling the direction of the rays from the sun, and concentrating them at the right point for illuminating the object. The heliostat is an instrument with clockwork construction, which causes the reflecting mirror to follow the sun, and thus project the rays into the tube of the microscope, requiring but little if any adjustment of screws on the part of the manipulator.

Electricity. — Although this source of light is not as bright as direct sunlight, still the author considers it to be preferable, for

the reason that it is possible to have it under thorough control. Moreover, the intensity is entirely sufficient for all purposes in the laboratory, and the use of sunlight for projection may be dispensed with.

Electricity is used in the production of two kinds of light, the incandescent and the arc. The latter is the most intense, but for a long time it was not practicable to use it for projection, for the reason that the carbons were so irregularly consumed it was impossible to keep the arc in the same position in the optical centre of the condensers. Not until within very recent time has this difficulty been overcome. To Messrs. J. B. Colt & Company is much of the credit due for devising an arc lamp which seems to satisfy all the demands reasonably made of such a light. The *Scientific American*, in speaking of this most excellent invention, says:¹—

“The lamp is perfectly automatic in the control of the carbons, which are disposed at such an angle as to present the crater of the positive carbon to the condensing lenses of the projector. This feature is very desirable, for, when carbons are vertical, even if the negative carbon is advanced toward the condenser out of line of the positive, the light will proceed from the negative carbon as well as the positive, thus making two sources of light instead of one—a condition fatal to definition on high-class work. If, however, the carbons are placed at an angle, as shown in the cut, the luminous spot on the negative carbon is obscured from the condenser, and the crater on the positive carbon is presented in a most favorable way. By an admirable system of mechanism in the lamp referred to, the point of the lower carbon is accurately maintained in a given position, and the upper carbon is gradually and regularly fed toward it in the exact proportion to its consumption. This mechanism is so simply and nicely adjusted that the lamp may be run for hours without a flicker, always maintaining the radiant in the optical centre of the projector.

“The regulating device is contained in a metal case, only 2

¹ *Scientific American*, January 20, 1894.

inches thick by $3\frac{1}{4}$ inches wide and $4\frac{1}{2}$ inches high, making it the most compact and easily adjusted lamp that has been brought to our notice.

“The negative carbon is automatically moved upward as it burns away. The positive carbon is fed down by the weight of the carbon holder and by a small spring-actuated train of gearing, which is held in check while the arc is of normal length, or released when the length of the arc becomes too great, by a shunt magnet contained by the casing. The action of the shunt magnet is controlled by a spring acting on its armature; an increase in the tension of the spring increases the length of the arc, while a reduction of the tension diminishes the length of the arc.

“The mechanism controlling the carbons is so constructed that the lamp may be used on from five to twenty amperes of current by the mere inserting of carbons of suitable sizes. The lamp is perfectly insulated, so it may be freely handled.

“This lamp may be clamped on a vertical post, with a sliding base, admitting of universal adjustment, or it may be mounted otherwise as may be desired. The utility of this lamp is by no means confined to optical projection, for, owing to its convenient size and the absolute steadiness of its light, it finds extensive use in photo-lithography and copying, micro-photography for theatrical effects, and, in fact, all uses for which an intense artificial light is desired. The intensity of this light admits of the use of the optical projector in a room that is only partially darkened, which greatly increases its utility and widens its scope.”

In Fig. 89, which is a representation of this lamp, *A* is a screw for raising or lowering the entire apparatus, so that proper centering of the light may be accomplished; *B* is a knob used in separating the carbons when the consumption requires the substitution of others; the magnets shown at *D* are for the purpose of separating the carbons when the current is turned on; *E* is the adjustment to enable the operator to place the lower pencil in the best position; *C* is a screw for adjusting the apparatus

laterally, while on the opposite side is a spring to hold the instrument firmly against *C*.

As in the case of other electrical appliances, a rheostat is required in the use of this lamp, and the one illustrated in Fig. 90 is suitable for low-voltage currents. This rheostat is made by the same firm who manufacture the electric lamp shown in Fig. 92.

Some suitable form of storage battery will be found convenient where a very steady light is demanded, although the lamp above

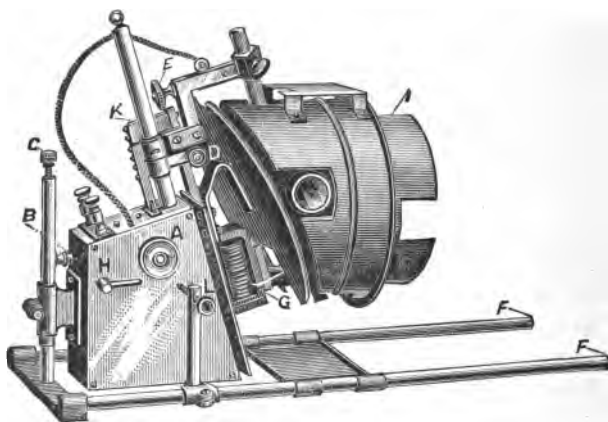


FIG. 89. — Colt's Electric Lamp.

illustrated will give a most constant flame from currents obtained directly from the dynamos. About thirty cells of the storage battery will be required to run the lamp. The current which comes either from the dynamo or the secondary battery must be direct and not alternating, and it should be from five to fifteen amperes and not less than sixty volts in pressure. A safety fuse should be placed in the circuit, so that if any accident occurs, by short-circuiting, the installation will not be injured. If a short-circuit takes place while the wires are being attached to the lamp, the safety fuse will be immediately burnt out before the instrument is affected. Inasmuch as the electromotive force of the current usually received directly from the dynamos

is 110 volts, it is necessary to introduce the rheostat or resistance coils (see Fig. 90) in order to give the correct voltage at the lamp terminals.

Before the lamp is lighted the two carbons are in contact, and as soon as the current begins to flow the ends of the carbons become heated, and the mechanism of the lamp separates them immediately, and the arc is at once established. The theory explaining this action is that electricity, at the usual pressure, will not pass through a space unless filled with the particles of the incandescent carbon. When the separation takes place, the arc is maintained by the vapor or particles of the highly heated carbon. In connecting up the wires to the lamp it must be remembered that the carbon containing the crater must be placed in connection with the positive pole of the dynamo, or battery, and the carbon containing the point must be joined to the negative pole. The first, or positive, pencil must be at the top of the lamp, and both pencils must be inclined, so that the light will not be projected downward, as will be the case if the carbons are kept in a vertical position, but thrown out directly in front along the optic axis. There is a limit, however, to this inclination, because, since the lower pencil is slightly in front of the upper, too much inclination will cause the point of the lower carbon to be projected on the screen in the form of a shadow. The best length to be given to the arc for steady results is from $\frac{1}{8}$ to $\frac{3}{16}$ of an inch. When it is too short, there is an unpleasant hissing and spluttering noise. In the lamp illustrated in Fig. 89, there is a mechanism provided for controlling the distance between the carbons. The positive carbon is consumed faster than the negative, and it is at all times kept in the form

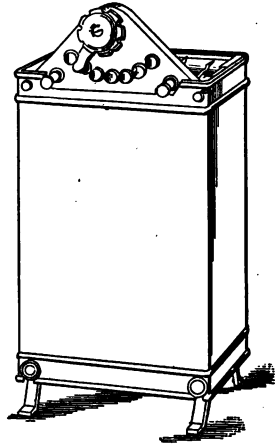


FIG. 90. — Rheostat.

of a crater, from the depths of which come the luminous rays that are projected into the lantern.

Oxy-hydrogen, or lime light. — This light is produced by the heating to incandescence a stick of lime under the combined action of burning oxygen and hydrogen gases. But little need be said, however, concerning this method of lighting, since the electric current is so readily secured in any well-equipped laboratory, and there is so little inconvenience and danger connected with its use. The oxy-hydrogen illumination, unless managed with the greatest care, is liable to give serious trouble, and there is always an element of danger associated with it, because of the explosive properties of the gases, which make its use in the hands of students unsafe. If, however, such a light is desired, the gases can be purchased compressed in metal cylinders ready for immediate use with but little cost. Or, these gases can be generated in the laboratory with but little trouble, provided the apparatus is secured. The oxygen is made by heating in a retort a mixture of one part of black oxide of manganese with four parts of chlorate of potash. Be careful to have the retort perfectly clean and thoroughly dry, and use only the purest chemicals. If they are pure, they will melt on an iron spoon over a lamp without an explosion. Pass the gas from a retort through a purifying-bottle and then into the metal cylinder. See that all connections are open and clear of all obstructions, and let the gas flow a short time before connection with the cylinder. All the air possible must be excluded from all parts of the apparatus to insure good results.

The hydrogen gas is generated by the action of sulphuric acid on metal zinc. Pass the gas through a purifying bottle, as in the case of the oxygen, and the same care must be taken in its preparation as indicated in the manufacture of oxygen, in order to prevent explosions and serious damage to person and property.

The following apparatus will complete the outfit for producing the calcium light : —

Oxygen and hydrogen cylinders.

Pressure gauges for the cylinders to measure their contents (see Fig. 91).

Light-regulator valves.

Oxy-hydrogen mixing jet for holding the lime pencil and mixing the gases before they strike the lime.

Hood for holding the lamp fixtures.

And, if the gases are made in the laboratory, generators, retorts, wash bottles, and rubber tubing.

Gas Lamp. — The best form is Welsbach's gas burner and reflector as devised by J. B. Colt & Company. This burner gives a strong light, and will answer very well where electricity or the lime light is not available. In fact, it will serve



FIG. 91.— Pressure Gauge.

most excellently in all cases where low-power objectives are used.

Acetylene-gas light. — There are several different kinds of lamps and generators on the market for burning and manufacturing acetylene gas, but all the generators are constructed upon the principle of immersing calcium carbide in water. Calcium carbide is a crystallization caused by fusing with an intense electric energy a combination of powdered coke and lime in proper proportions. The gas is generated by bringing the calcium carbide in contact with water, when immediately a colorless gas is generated which is unaffected by variations of temperature. The

light given by this gas is very bright and pure in color, and is very much like the light received from the sun. The carbide is very cheap, costing less than $3\frac{1}{4}$ cents per pound, and the increased use of the gas will make it still cheaper. ¹ One pound of the carbide will develop about five cubic feet of gas.

Calcium carbide, when in the solid form and properly stored and protected from contact with water, is non-explosive. The greatest precaution, however, must be used in the manufacture of the gas because of its explosive properties, but it is perfectly

safe, if due care is taken in placing the carbide in the generators to see that fire is not brought in range of the apparatus, and that there are no leaks in the pipes conducting the gas to the burners.

The burner has a peculiar shape, differing from that given to the burners used with the ordinary city gas. The illustration shown in Fig. 92 represents the usual form adopted for these burners. This is the one used by J. B. Colt & Company with their acetylene generators.

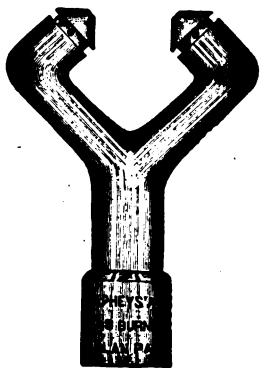


FIG. 92. — Colt's Acetylene Gas Burner.

Oil light. — Sometimes it is impossible to obtain either the electric, the oxy-hydrogen, the acetylene, or the gas light, and under these circumstances a good oil lamp will render efficient service, if the light is of that nature furnished by a well-conditioned student's kerosene-oil lamp. Of course it must be remembered that the light from such a source is by no means as brilliant as that secured from either of the lamps already described, and that the exposure will be correspondingly long in making the photo-micrographs with kerosene-oil light.

The author has seen some beautiful photo-micrographs made by the oil light with the high powers of objectives, but he well remembers that the work was done by the hands of skilful operators.

CHAPTER XII

PHOTO-MICROGRAPHS (CONTINUED)—PHOTOGRAPHIC DRY PLATES AND MAKING THE NEGATIVES

THE student should begin with the low-power objectives first and not attempt too difficult work until he has mastered the simplest details. The arrangement of the apparatus must be carefully looked after and the parts all accurately adjusted so that all of the glasses will occupy positions in the same optic axis. There must not be the slightest leak of light in any portion of the outfit. It is best to use with the objective the projection ocular described on page 35, although good work has been done in the past by some of our leading microscopists by the use of the objective alone. Only the best glasses must be used in this work, because it will be found very difficult to make the actinic and visual foci coincide. With the use of the Jena apochromatic lenses, however, this difficulty is almost entirely overcome, and it is possible to secure objectives which are specially made for use with the photo-micrographic apparatus, with which when the visual image is focussed on the ground glass an image equally sharp in its actinic effects is projected on the sensitive surface of the dry plate.

Mr. T. Comber, in the *Journal of the Liverpool Microscopical Society*, gives the following steps for adjusting the photo-micrographic apparatus with sunlight as the illuminant:—

“1. Accurately centre the achromatic condenser, regulating the diaphragm so that the opening is a little smaller than the field.

“2. Remove the mirror from the heliostat, to ascertain that the spindle appears precisely on and in the field, which it should do if the heliostat has been properly placed. Exactness

in this adjustment is necessary, otherwise the beam of sunlight will not be motionless. The mirror is then replaced to reflect the sun in the centre of the field. At this stage the eye must be protected by a dark-colored glass placed below the condenser.

"3. The object being placed on the stage is brought into the centre of the field and focussed.

"4. The condenser is next focussed to throw the sun's image exactly in the plane of the object. Sharpness of the ultimate image upon the ground glass cannot be secured without this.

"5. Changing the objective to one-sixth (4 mm.), I next measure the thickness of the cover-glass, or rather the distance between that plane of the object which it is desired to photograph and the upper surface of the cover-glass, by means of the fine adjustment screw. The purpose of this is twofold: 1st, To facilitate cover correction; 2d, To ascertain whether the 2 mm. object-glass, which is now put on, can get down to it, for its front lens is rather more than a hemisphere. . . .

"6. The illuminating cone thrown by the condenser has to be regulated. The width of the cone should vary according to the nature of the object and the quality of the object-glass. Too narrow a cone produces diffraction fringes, that bane of photo-micrography; too wide a cone produces haze. To get true images, the cone, whether it be wide or narrow, must be absolutely axial. Even a very slight obliquity renders the images unreliable.

"7. The ordinary eyepiece is now changed for a projection eyepiece, set at the distance at which the sensitive plate is to stand; the camera is attached and the long focussing rod coupled on (see Fig. 88). The image of the sun will be found in the centre of the ground glass. If it is not, the centring of the condenser must be wrong, and will require alteration. The sun's image should be sharp at the edge, unless the sky is hazy. The image of the object, as seen against that of the sun, will be somewhat out of focus, but a slight turn of the focussing rod brings it right."¹

¹ See also *Four. Roy. Mic. Soc.*

The instructions contained in the above copy from the article by Mr. Comber will also apply when the electric lamp is used in place of the sun. Proceed in the same manner in all respects while focussing, except that the electric lamp is substituted for the heliostat.

Since it is the common practice of microscopists to stain the sections, in order that distinctive contrast in the systems of cellular structure may be secured, it will be found difficult to produce good negatives, presenting clearness in detail, with the use of the ordinary dry plate. It has been found necessary, therefore, to submit the plates to a special treatment, so as to correct them for color values. The student can prepare the ordinary dry plates by immersing them in erythrosin and cyanin solutions. When so treated the plates are called *orthochromatic*, and they may be purchased from dealers, sensitized for all the colors of the spectrum. A most excellent account of the method for preparing the plate is given in Anthony's *Photographic Bulletin for 1893*, p. 608, extracts from which are given below. The article from which these items were gathered was read before the Société Française de Photographie, on May 5, by M. Monpillard.¹

"Orthochromatic sensitiveness.— Though chemical orthochromatic plates are sensitive for the green and red, and generally give satisfaction, M. Monpillard says that, for scientific purposes, he prefers ready orthochromatized plates, which, when used shortly after preparation, have a maximum of sensitiveness to the luminous radiations. The operation of orthochromatization demands only elementary care. The dark-room lamp should have two thicknesses of deep ruby glass, the flame being reduced to as small a degree as convenient during the bathing of the plates. After the plates are bathed they are passed through three dishes of distilled water, and are finally dried in a drying cupboard containing a vessel in which calcium chloride is placed.

"For photo-micrographic purposes the following colors give

¹ See also *Four. Roy. Mic. Soc.* for February, 1894, p. 113.

the best results: (1) Erythrosin (for green-yellow, yellow, and yellow-orange); (2) Cyanin (for red-orange and red).

"M. Monpillard says the following formulæ have given him satisfaction:—

"Erythrosin (stock solution): Erythrosin, 1 part; distilled water, 1000 parts.

"Sensitizing bath: Stock solution of erythrosin, 4 cc.; water 100 cc.; ammonia, 0.5 cc.

"Cyanin (stock solution): Cyanin, 0.1 part; alcohol (95 per cent), 100 parts. Only a small quantity of the solution should be prepared, and it should be kept in the dark.

"Sensitizing bath: Stock solution of cyanin, 4 cc.; water, 100 cc.; alcohol (95 per cent), 5 cc.; ammonia, 1.5 cc. The plates are immersed in either of the foregoing baths for two minutes, and are then washed and dried as directed.

"Erythrosin and cyanin plates bathed in both erythrosin and cyanin are rendered sensitive to yellow and red. The first bath consists of: Stock solution of erythrosin, 20 cc.; distilled water, 80 cc. After two minutes' immersion, the plates are washed in two waters, and are then bathed in the cyanin solution given, washed and dried.

"Plates so treated are, it is pointed out, very much slower, but this is no disadvantage in photo-micrography, and on the other hand they do not fog in development, which frequently happens when, to raise their general sensitiveness, the orthochromatizing bath is preceded by an alkalin bath.

"**Colored screens.**—Colored screens may be used either in the form of stained collodion, or, preferably, a small glass trough, with parallel faces, may be fitted with either of the following solutions:—

"(1) For light yellow screen: Neutral chromate of potash, 1 grm.; water, 100 parts.

"(2) For deep yellow screen: Neutral chromate of potash, 5 grms.; water, 100 parts.

"(3) For orange screen: Bichromate of potash, 8 grms.; water, 100 parts.

"(4) For red screen: Erythrosin, 0.2 grm.; water, 100 parts.

"Number 1 weakens the blues and yellows; No. 2 extinguishes them; No. 3 cuts off the blue; No. 4 accentuates the action of the red.

"With these colored screens, and having sensitized the plates for given colors, it will be easy to obtain, in their true values, reproductions of objects, colored or uncolored. It is necessary, however, that the focus and the exposure should be made in the same monochromatic light, corresponding to a determined spectrum color. This method of working assures the perfect sharpness of the image, inasmuch as the chemical focus is corrected.

"Where an object combines red and yellow colors, . . . it would be better to sensitize for red and yellow, and, according to the intensity of the former, expose with a deep yellow, or orange, screen. If blues and violets are found in the presence of yellows, oranges, or reds, it would suffice to use a plate sensitized for the least actinic color (yellow or red), and as the plate is, of course, sensitive to the blues and violets, a yellow screen, pale or deep, could be used according as the more actinic parts of the object are more or less colored. For development, the author recommends hydroquinon with an alkalin carbonate and bromide, and the use of a feeble light in the dark room."

The color screen can be placed in the front of the camera, in such a position that all the rays coming from the object must pass through it before reaching the sensitive dry plate.

Exposures. — There are so many elements controlling the degree of exposure that it is quite difficult to put down any fixed rules to guide the operator. The character of the light, the power of the objective used, and the condition of the object, are all to be well understood before this question can be determined, and these factors can only be known after careful experiment. As a general guide it may be stated that with orthochromatic plates used in connection with the color screen the following

figures give about the limits of exposures. These results have been determined by Mr. W. H. Walmsley, who has done some excellent work in photo-micrography. He used, however, a coal oil or petroleum lamp to supply the illumination, and in the substitution of the sunlight or electric light the time will be considerably reduced.

$1\frac{1}{2}$ -inch objective, 3 to 45 seconds.

$\frac{3}{4}$ -inch objective, $\frac{1}{4}$ to $1\frac{1}{2}$ minutes.

$\frac{1}{10}$ objective, $\frac{1}{2}$ to 3 minutes.

$\frac{1}{8}$ objective, 2 to 7 minutes.

$\frac{1}{10}$ objective, 5 to 10 minutes.

The sensitive plate having been carefully dusted and placed in the plate-holder — which, of course, must be done in the dark room where no ray of white light can strike its surface — and the focussing of all parts of the apparatus having been thoroughly accomplished, as already explained, the exposure is made by attaching the plate-holder to the camera in the place of the ground glass. The light is first excluded from the apparatus by placing an opaque card with a black surface between the objective lens and the stage, or, if very high powers are used, immediately beneath the stage. The slide is now withdrawn from the holder, and, with watch in the left hand, the card is quickly raised, the light permitted to enter the instrument and project the image of the object on the sensitive surface of the plate. After the exposure is completed quickly return the card to its place, and then push in the slide. Now unfasten the holder from the camera, and return with it to the dark room for the purpose of developing the picture.

Before opening the holder to take out the plate prepare the following developing and fixing solutions. As a matter of illustration we will take Carbutt's orthochromatic plates to show the method of treatment, and his formulæ are given, therefore, in these instructions; but the student will find at the close of this volume other developers if he desires to use plates made by different manufacturers.

Eikonogen and Hydrochinon Developer

A

Distilled water	600 cc.
Sulphite of soda crystals	120 grammes
Eikonogen	22 grammes
Hydrochinon	10½ grammes
Water to <i>make up to</i>	960 cc.

B

Distilled water	600 cc.
Carbonate of potash	60 grammes
Carbonate soda crystals	60 grammes
Water to <i>make up to</i>	960 cc.

	A	B	Water
For instantaneous exposures	30 cc.	30 cc.	120 cc.
“ portraits	30 “	30 “	150 “
“ landscapes { Sen. 20-27 }	30 “	15 “	90 “
Full exposures { “ 16-20 }			
“ lantern slides	30 “	25 “	120 “
Full exposures	30 “	25 “	120 “

Add 2 to 6 drops restrainer D to each ounce of developer for lantern slides and full exposures.

NOTE. — More of A will increase density, more of B will increase detail and softness. The temperature of the developer should not vary much below 18.3° C. nor above 24° C.

Restrainer

D

Bromide potassa	14 grammes
Water	150 cc.

Select a suitable tray for the size of the plate, take the plate from the holder, and place it sensitive side up in the tray, and pour over it *quickly* the developing solution. Now gently rock the tray back and forth, so that fresh portions of the liquid will

strike every part of the surface, as is shown in Fig. 74. If the plate has been properly exposed, the image will begin to show within fifteen to thirty seconds; but if it has been exposed too long to the action of the light, *overexposed*, as it is called, the image flashes up very soon after the developer strikes the surface, and then rapidly sinks into the film and blackens all over the surface. If an *underexposure* has been made, the image fails to appear until after a minute or more has passed, and the details are flat and faint, the ground of the resulting negative being a sickly grayish tint. In the case of a very much overexposed or underexposed plate it is best to try again, and repeat the operation until the correct exposure is approximately attained.

After the details of the picture have come out well, and the image begins to sink gradually into the film, the developing action is stopped by pouring the solution back into the graduate, and washing the plate under the water-tap. It must be remembered that the orthochromatic plate is very sensitive, and exposure for a time in any light which is safe for the ordinary plates will, after a short time, cause the negative to fog. The development must, therefore, be almost entirely accomplished with the tray covered. The plate is now placed in the fixing-bath, and permitted to remain under the action of the solution until all the soluble salts of silver are dissolved out and no white cloudiness is perceptible on the back of the plate. The following is the method of preparing the fixing solution:—

Carbutt's Acid Fixing and Clearing Bath

Sulphuric acid		4 cc.
Hyposulphite of soda		480 grammes
Sulphite of soda		60 grammes
Chrome alum		30 grammes
Warm water		1920 cc.

During cold weather use only half the quantity of chrome alum given above.

“Dissolve the hyposulphite of soda in 48 ounces (1440 cc.) of water, the sulphite of soda in 6 ounces (180 cc.) of water,

mix the sulphuric acid with 2 ounces (60 cc.) of water, and pour slowly into the sulphite of soda solution, and add to the hyposulphite, then dissolve the chrome alum in 8 ounces (240 cc.) of water, and add to the bulk of solution, and the bath is ready. This bath will not discolor until after long usage, and both clears up the shadows of the negative and hardens the film at the same time."

When the negative has been fully treated with the fixing solution, it may be taken out into white light without any further danger of injury. Before exposing to white light all the soluble silver must be dissolved by the fixing-bath, which is indicated by the disappearance of the white cloud seen from the back of the negative. In a fresh fixing-bath this will require about ten minutes. Before drying it is thoroughly washed in running water for at least a half-hour until all of the hyposulphite of soda is eliminated from the film. This washing is most conveniently done in the washing box illustrated in Fig. 82. The prolonged washing is necessary because the hypo salts hold on to the film with such tenacity, and, unless entirely extracted, the negative will soon become yellow and lose its brilliancy and value.

After complete washing, the plate is swabbed with a wad of wet cotton and placed on edge in the drying-rack to dry spontaneously (see Fig. 84). The drying will take place much sooner if the rack is situated in a draught of cool air. Care must be exercised, however, to prevent heat reaching the plate, but the drying must be in the shade and in cool air, otherwise the film will melt and the negative be destroyed. When thoroughly dried, the surface of the negative is coated with a varnish to protect it from the effects of the printing processes while making the positives. A permanent number is then made on one corner of the plate, and a corresponding number is also placed on the envelope or preserver, which contains the name of the object, date, and such other data as may be desired for future reference. If the negative requires intensification, as is sometimes the case, the following method is recommended by Carbutt, and is most excellent:—

Carbutt's Intensifying Solution

No. 1

Bichloride of mercury	16 grammes
Chloride of ammonia	16 grammes
Distilled water	600 cc.

No. 2

Chloride of ammonia	16 grammes
Water	600 cc.

"Flow sufficient of No. 1 over the negative to cover it, and allow either partially or entirely to whiten; *the longer it is allowed to act the more intense* will be the result; pour off into the sink, rinse, and flow over No. 2, and allow to act one minute; wash off and pour over or immerse in dilute ammonia water (11 cc. of strong ammonia in 240 cc. of water). In place of ammonia a 10 per cent solution of sulphite of soda may be used until changed entirely to a dark brown or black. Wash thoroughly and dry."

If the celluloid films are used for making the negatives instead of the glass plates, the method of procedure is the same, except that when the last washing has been accomplished, the films are soaked for five minutes in the following solution in order to cause them to remain flat and not curl up:—

Water	750 cc.
Glycerin	30 cc.

It often becomes necessary to touch up the negative in order to remove from it any defects that occur at times in the film, and this must be done before the final finishing varnish is applied. For retouching, either graphite pencils or some opaque paint such as Gihon's is used. The best pencils for this purpose are L. & C. Hardtmuth's (Vienna) "black chalk points." Figure 85 shows a good retouching-frame for holding the negative and directing the light during the operation of pencilling out the defects.

The equipment of the dark room.— Besides the items mentioned on page 12, the dark room should be provided with developing pans, or dishes, or developing trays, of the following sizes :—

$$\begin{array}{ll} 3\frac{1}{2} \times 4\frac{1}{2} \text{ inches.} & 4\frac{3}{8} \times 5\frac{5}{8} \text{ inches.} \\ 4\frac{1}{2} \times 6\frac{3}{4} \text{ inches.} & 5\frac{1}{2} \times 8\frac{1}{2} \text{ inches.} \\ & 7 \times 9 \text{ inches.} \end{array}$$

These trays must always be kept in convenient reach near the bottles containing the developing solutions, and they should be used for no other purposes. The best trays are made out of hard rubber (Fig. 79), with two grooves running the length of the tray, upon which the plate can rest, and thus furnish a convenient means of raising the negative when it is desired to examine the progress of the developing.

On another shelf of the dark room, sufficiently removed from the developing shelf, or table, to avoid all chance of mixing the chemicals, the hard rubber fixing-baths to contain the hyposulphite of soda solutions may be located (Fig. 81). The following sizes will be found suitable for most purposes :—

$$\begin{array}{ll} 3\frac{1}{4} \times 4\frac{1}{4} \text{ inches.} & 4 \times 5 \text{ inches.} \\ 5 \times 7 \text{ inches.} & 5 \times 8 \text{ inches.} \\ & 6\frac{1}{2} \times 8\frac{1}{2} \text{ inches.} \end{array}$$

These fixing-bath vessels are formed in such a manner as to hold the plates in a vertical position, and the grooves will permit one dozen negatives to be fixed at the same time. The sediment which may accumulate is kept from contact with the soft film by a ridge rising above the bottom of the vessel.

Several graduates, or measuring glasses, of 4-ounce or 120 cc. capacity must also be in the dark room for enabling the operator to secure the proper proportions of the developing agents (Fig. 80).

A minim glass will also be essential.

The light used in the dark room must be carefully covered with ruby glass and also two thicknesses of yellow paper, or

glass, to insure the proper protection of the plates while transferring them to the plate-holder and developing. If electricity is not available, a gas lamp may be used, or Carbutt's "Multum in Parvo" is an excellent lamp (Fig. 77). This lamp has three doors; in one is a 2×2.5 dm. plate of ruby glass; in the second is a plate of opal glass, covered with a metal door, which is used for examining the finished negative; and the third opening contains a clear glass through which the white light comes when the lamp is used for contact printing in making lantern slides.

Development pointers. — "1. Development containing more alkali produces more detail in the shadows and lessens intensity of the high lights, which causes more softness in the negative. Suitable for underexposed plates.

"2. Extra quantities of pyrogalllic acid, eikonogen, metol, or hydrochinon quickly intensify the high lights.

"3. Developer diluted with water slows the action of the chemicals and gives the shadows more chance to work through before the high lights have gained their strength, and is recommended for overexposed plates.

"4. There is no such thing as perfectly safe light, and the illumination in the dark room must be carefully looked after. The best and safest light for ordinary plates and the eyes is a combination of ruby and orange glasses.

"5. Dust the plate slowly, so that electric currents may not be generated and the dust become electrified and render the cleaning of the surface almost impossible.

"6. Thorough fixing, thorough washing followed by quick drying in the shade, will insure permanency and fine printing qualities.

"7. Do not expose the negative to white light until all trace of the bromide of silver has disappeared from the back. As long as there is any white cloudiness on the back keep the plate in the hyposulphite of soda bath."

Defects in the Negative. — The following points will be serviceable to the beginner in determining what is the matter with an inferior negative and what steps are required to correct the matter: —

"1. Foggy. Caused by overexposure; white light entering the camera or the dark room; too much light during development; decomposed chemicals used in development; introduction of hyposulphite of soda into the developer; developer too warm, or containing too much carbonate of soda or potassium.

"2. Weak with clear shadows. Underdevelopment.

"3. Too strong with clear shadows. Underexposure.

"4. Weak with plenty of detail in shadows. Want of intensity caused by overexposure. Shorter exposure with longer development will, in most cases, produce intensity, and an addition of more stock solution to development will seldom be necessary.

"5. Fine transparent lines. Too stiff brush in cleaning plates before placing in the plate-holder.

"6. Transparent spots and pinholes. Dust on the plate or in the camera, or scum on old developer, or air bubbles while developing. *Developer must be clean.*

"7. Crystallization on negative and fading of the image. Hyposulphite of soda not well washed out of film after fixing.

"8. Yellow negatives. Not using enough sulphite of soda, or the article is old and decomposed.

"9. Mottled negative. Precipitation from fixing-bath containing alum, if the solution is old and turbid."

CHAPTER XIII

MAKING THE POSITIVES

A POSITIVE is just the reverse of the negative and represents the view in its complete and natural condition. Where the shading is shown in the negative by the deposit of the silver salts, the white and the gradations into the shadow are given in the positive. Perfectly clear glass on the negative will give deep shadow on the positive. If therefore a first-class negative is secured it is not a difficult matter to make a first-class positive from it, but if the work of exposing the sensitive plate and its development has not been skilfully done the negative is often too poor to spend any time in making positives from it, and it would be best to expose another plate for another and better negative, if the object is near and of easy access. Sometimes, however, it is impossible to make another exposure on the special view and we are compelled to attempt to make our positives from an inferior negative or forego having pictures of the scene. Under these conditions some little ingenuity and skill must be exercised to obtain a picture that is even passable. There is no excuse, however, for using an inferior negative in photo-micrographical work, because the student can readily repeat his work until he does obtain a good negative, since the apparatus and the object are located near the dark room and the question of time simply becomes the matter for consideration.

Photographers recognize two chief kinds of positives : —

1. Those made on paper and cloth.
2. Those made on glass.

The first class may be subdivided into : —

1. Plain and albumenized paper, sensitized with silver salts. These papers are known in the market by such names as "Disco,"

"Solio," "Aristo-platino," "W. C. Platinotype," etc. These are termed by photographers "printing-out papers." They are made by exposing the sensitized paper under the negative to the sunlight and then afterward treating them with chemicals to fix the picture and tone it to the desired color.

2. Slow bromide, or developing papers. These are made by exposing the sensitized paper under the negative before a gas or other lamp light and then developing the picture with the same chemicals and in the similar manner as in making the negative, until the image comes out and is fixed on the paper in the tone desired. The trade names for these papers are "Argo," "Dekko," "Nepera," "Velox," "Vincos," etc.

3. Ferro-Prussiate, or blue papers.

The second class may be divided into:—

1. Lantern slides.

2. Window transparencies.

To make positives on the albumenized paper, proceed as follows. This paper already sensitized may be purchased from any first-class dealer in photographic goods, but it may also be made in the laboratory if used up within a few hours. It is difficult to make this character of paper so that it will keep for several months, but the brands made for sale are manufactured under a secret formula which preserves the sensitive surface for some time.

Select the proper size of printing-frame (see Fig. 83) to suit the size of negative, place the negative in it with the film side up, and place the paper in contact with its sensitive surface in touch with the film of the negative, attach firmly the back of the printing-frame and expose the glass side to the light reflected from the sky, if the negative is weak, or if very strong to the direct light from the sun. Permit the action of the light to continue until the picture comes out slightly deeper than the finished picture should be, because the treatment with the chemicals will somewhat fade the image and allowance must be made for this. Putting the sensitive paper under the negative must be done in a portion of the room where the light is not very

strong, and when the back of the printing-frame is raised to examine the progress of the printing turn your back to the light and expose the paper off of the negative as short a time as possible.

When the printing has been carried far enough, take the paper out of the frame and immerse in a vessel of water for a minute so that a portion of the soluble silver salts may be dissolved out, and then transfer to the following toning-bath:—

Chloride of gold	1 grain (0.06 gramme)
Acetate of sodium	30 grains (2.0 grammes)
Water	8 ounces (240.0 cc.)

This solution must not be used at once but must be allowed to stand for about 24 hours. Let the prints remain in the toning-bath until they assume the tone desired and then place them in the fixing-bath, which is prepared as follows:—

Hyposulphite of soda	2 ounces (60 grammes)
Water	48 ounces (1440 cc.)

Fixing in this bath for about fifteen minutes will suffice to make the picture relatively permanent. Do not permit the prints to come in contact with each other while fixing, but it will be best to keep them in motion for the finest results. After fixing wash them in running water for half an hour, or in six changes of water after soaking in each change for five minutes. It is absolutely necessary that the hyposulphite of soda be entirely eliminated from the fibres of the paper, or otherwise the print will soon fade and turn yellow. Dry after washing thoroughly by spreading the papers on clean blotting-paper with the picture *up*, and then store them away until ready to mount on the cardboard. If the mounting on the card is to be done at once proceed as follows: Trim the prints to the size and shape desired and place them on a clean glass plate with the picture side down, one print above another, until all have been trimmed and thus transferred from the water to the glass plate. Press out the water with the hand or a rubber roller, such as is used

by photographers, and rub over the top print a thin film of paste free from all lumps and solid matter. Catch the paper at opposite corners and bring in contact with the card, permitting the centre of the paper to touch first and then lowering each of the corners until the entire portion of the print is in contact with the card. Place a blotter over the print and press into close union with the card by moving the fingers from the centre to the edges of the paper. Or use a rubber roller which is made for the purpose. When the mount is dry the paper will have contracted and all wrinkles will disappear and the surface of the paper will be smooth.

The method used in making the positives with the developing papers mentioned under the second subdivision on page 187 is somewhat different from the one just described. These developing papers are much more rapid in their work than the printing-out papers, because the surfaces are more sensitive to the action of light. We therefore expose them before a lamp instead of the direct sun rays. The same kind of printing-frame is used with these as in the case of the papers just described. Do all the work in the dark room with ruby light until the papers are placed under the negatives and until ready to make the exposure. Stand the printing-frame a short distance from the lamp, the distance depending on the character of the negative and the strength of light, and then permit white light to fall on the glass side of the negative for a few seconds, from ten to thirty, depending also on the density of the negative and strength of light. Cover the white light and proceed to develop the picture in the same manner as that used in making the negative, viz.: Place the sensitive paper after exposure in a developing pan with the sensitive side up and flood over it a developing solution recommended by the manufacturer of the paper, and permit this developer to act until the picture comes out clear and slightly deeper in shade than it should be when finished, and then fix in the hyposulphite bath for ten or fifteen minutes. Wash for a half-hour in running water to extract all the hyposulphite of soda and then mount in the manner already described

above. To illustrate this method of making positives with the developing papers we will take the "VincO" through the process.

Expose before a gas lamp, in the manner described above, and develop in the following solution :—

Water	600 cc.
Metol	2 grammes
Hydrochinon	$\frac{2}{3}$ grammes
Sulphite of soda (granulated)	7 grammes
Carbonate of soda (granulated)	7 grammes
Potassium bromide	$\frac{1}{16}$ grammes

If the negative is vigorous dilute with one-half water ; for soft detail negatives shorten the exposure and use the full strength of the developer. After the picture comes out to the degree of shade slightly beyond that required for the finished picture, arrest the development by placing the print in the following short stop and hardener :—

Table salt	30 grammes
Powdered alum	30 grammes
Water	960 cc.

One minute in this solution will suffice ; rinse and transfer to the fixing-bath :—

Hyposulphite of soda	240 grammes
Sulphite of soda (granulated)	30 grammes
Sulphuric acid	4 cc.
Water	960 cc.

Dissolve the sulphite of soda and hyposulphite of soda in separate vessels and add the sulphuric acid to the sulphite of soda solution and then mix the two solutions together.

Permit the prints to remain in the fixing-bath for fifteen minutes, and then bring out in the white light and wash in running water for a half-hour until all the hyposulphite of soda is eliminated. During the fixing keep the prints separate so that discoloration will not take place.

Lantern Slides: How to make them, and the Use of the Lantern for projecting Photo-micrographs on the Screen

The sensitive plates, used for making the lantern slides, are coated with a gelatin bromide emulsion which, when exposed to light through a negative, and developed according to directions, will give an attractive positive firmly adhering to the glass. Lantern slides of a high grade of excellence may also be made by the use of the "wet plates," which of course must be coated with the sensitive emulsion just before using. This is the old method, and is well understood by the professional photographer, and was the only method for making slides before the "dry plate" came into such general use. It requires some experience, however, to prepare the plate properly, and there is more trouble in manipulating it in the hands of the amateur. When carefully handled the dry plates give brilliant and fine results, and they are to be recommended to the student.

The dry plates are sent out by the manufacturers in light-tight boxes, one dozen in each box. The operator should obtain a negative box the proper size, and fill it with the plates after they have been dusted with a camel's-hair brush. This transfer must, of course, be done in the dark room. The negative box will hold twenty-four plates.

The lantern slides are made by either one of the following methods:—

1. The contact method.

2. Photographing the negative by means of a camera.

The first method is only feasible when the negative has been made near the size of the picture to be transferred on the positive glass, and the work is accomplished as follows: Mix a sufficient quantity of the developer, in accordance with instructions contained on page 179, light the lamp, and exclude all rays from the dark room except those which come through the ruby glass of the lamp. Place a negative, film side up, in a deep printing-frame, take one of the sensitive plates from the box and lay it on the negative *with film in contact with film*, place a

piece of thick felt on the back of the sensitive plate to exclude all reflected rays of light from the rear of the printing-frame, and then put on the hinged back and clamp it into place. Now stand the frame on edge about 4.6 or 6.1 dm. from the lamp with the negative toward the light, cover the frame with an opaque cloth, or interpose a black card, and then open the door of the lamp, so that the white light may be projected toward the printing-frame, raise the cloth or cover for a few seconds (the time depending upon the distance between the light and negative, strength of light, and density of negative) until the sensitive plate is sufficiently affected, when the light is cut off. The plate is now transferred to the developing pan with the film side up, and the developing solution quickly poured over it. If the picture comes up rapidly, the exposure has been too long, and another trial should be made. There is but little advantage gained in trying to doctor an underexposed or very much overexposed plate. It is cheaper, and best in all respects, to expose another plate, either prolonging or shortening the time of exposure, as the case may demand, until the image begins to make its appearance, not sooner than twenty seconds, and continues to come out gradually and slowly. The development must not be stopped until the high lights just begin to tinge, and all the details of the picture are well out, and distinct contrasts exist between the high lights and deep shadows, with gradual blending between them. The plate is now washed and placed in a fresh fixing-bath prepared according to the formula given on page 180. It must remain in this bath until all the white cloudiness has disappeared entirely from the back, when the plate can be taken out into the white light and examined with safety. The positive is considered to be first-class if it will show by transmitted light, when held up between the eye and the window, perfectly clear glass for the high lights and passing by imperceptible gradations to the deeper shadows, and if the tone is of a warm purplish black. The deep shadows must not be so opaque as to prevent the outlines of the flame of a lamp from being seen when the slide is held between the observer and the light.

Carefully wash the slide in the same manner described for the negative and swab off gently with a wad of soft cotton to take off all extraneous particles which may be adhering to the film, and stand in a rack to dry.

To make the slide by the second method, or photographing through the camera, it is necessary to have a camera with an extra-long bellows. The negative is suspended in front of a window with a northern exposure, if possible, and the camera is carefully adjusted at a suitable distance from the negative, so that the image projected on the ground-glass plate will be the desired size for the slide. The front of the camera should be parallel with the surface of the negative, and the optical axis of the lens must strike the centre of the picture. If the negative is smaller than desirable, reverse the lens of the camera, and the enlargement of the image is produced on the ground glass. A little ingenuity in manipulating the apparatus will soon produce the results sought for by the operator. As soon as all portions of the apparatus have been properly adjusted, the distance between the negative and the camera is covered with a dark opaque cloth to exclude all light except that which comes directly through the negative, and the operator is then ready to substitute the plate-holder containing the sensitive plate for the ground glass and make the exposure. Before pulling out the slide covering the plate, see that the lens has the proper diaphragm and is covered with a cap. The experience of the author has demonstrated that the diaphragm f 16 and the light coming from a clear northern sky, with Carbutt's "gelatino-albumen" plate, fifteen to twenty seconds, will yield brilliant lantern slides. There are the same factors, however, to be considered here which were mentioned in the case of the time of the exposure in making the negative. (See page 177.) The experience of the operator must determine these questions after he has become familiar with the qualities of his negative and the character of the light.

The after treatment of the plate is, in all respects, the same as that described under the first, or contact, method. When

the slide is thoroughly dry after washing, the film should be covered with a suitable varnish made for the purpose (usually colorless gums dissolved in benzol), so the moisture cannot

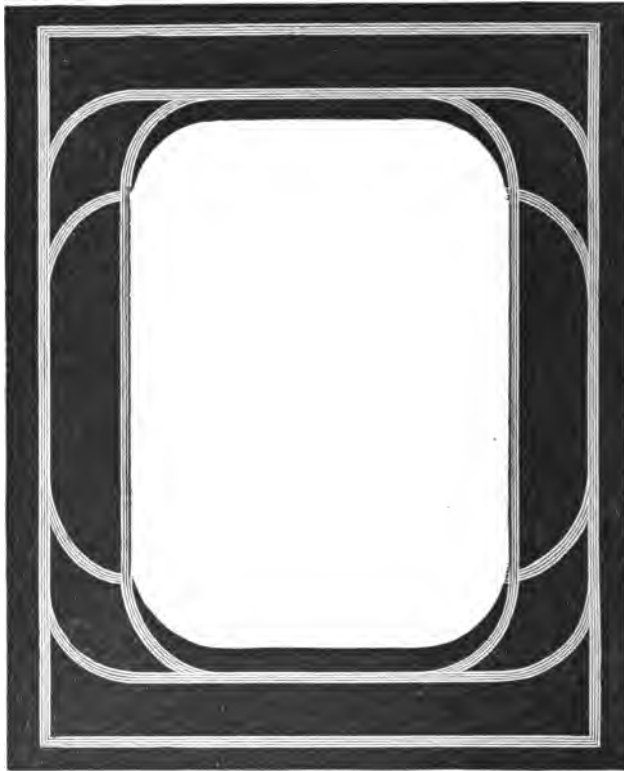


FIG. 93. — Mat for Lantern Slide.

injure the slide in the future. Moreover, the varnish benefits the slide in cleaning it up and brightening the picture throughout.

To mount the slide for permanent preservation, take a thin glass plate, perfectly free from all flaws and well cleaned, and cover the film. Between these two glass plates insert the mat illustrated in Fig. 93, and then bind the plates together by means

of a narrow strip of gummed paper three-eighths of an inch wide and fifteen inches long. Lay this strip on the table and wet with a dampened sponge three or four inches at one end. Take the slide, with the covering glass and mat in position, and press one of the edges in contact with the wet slip; invert the slide, resting the opposite edge on the table, and, by means of the fingers, bring the damp portions of the slip in close contact with the glass. Proceed to dampen the gum and adhere the slip



FIG. 94. — Box for Lantern Slides.

until all four edges of the slide have been covered. While performing this operation keep the fingers dry, if good work is desired. The label is placed on, and then the slide is ready for the lantern.

Figure 94 is an excellent box for holding the slides while they are being put through the lantern, and it will also serve a fine purpose in preserving them from the action of dust when stored away.

The lantern and its requisites. — The lantern consists of the following parts, which are absolutely essential to its well working:—

1. The radiant or lamp.
2. The condensers, for collecting or concentrating the light on the slide.
3. The slide carrier.
4. The body of the lantern, which consists of a metal box or an extension bellows.
5. The projecting system or lens for projecting the rays which pass through the slide on to the screen and produce the enlarged picture.

The description of the light suitable for the lantern has already been given in Chapter XI, so that the student is referred to that part of the book for information on this point. What has been said there in reference to illuminating the object for microphotographing is equally true in regard to the character of the light for projection through the lantern.

There are several first-class lamps, both oxy-hydrogen and electric, on the market which have been especially designed for the lantern, and the principles governing them being so nearly the same, the description of one will give a clear idea of the others. On page 168 is illustrated and described a lamp which the author has been using for several years with considerable satisfaction, for the micro-photographic outfit and for the lantern. This lamp, or one like it, will be found indispensable in the laboratory when it is desired to project on the screen a slide of the object which is being studied, either in the shape of a photograph on glass or a projection of an image direct from the object itself, by means of the microscope attachment to the lantern. Figure 95 is an illustration of how this application of the electric lamp and the lantern with the microscope attachment may be used. The object is placed on the stage at *a* and the lamp *b* transmits the light through the lantern *c*, to the object, and the objective *o* magnifies the image and sends it to the lens *d*, where it is projected to the screen greatly enlarged.

The oxy-hydrogen lamp is made to attach to this outfit in the same manner, if it is desired to use the lime light instead of the electric light.

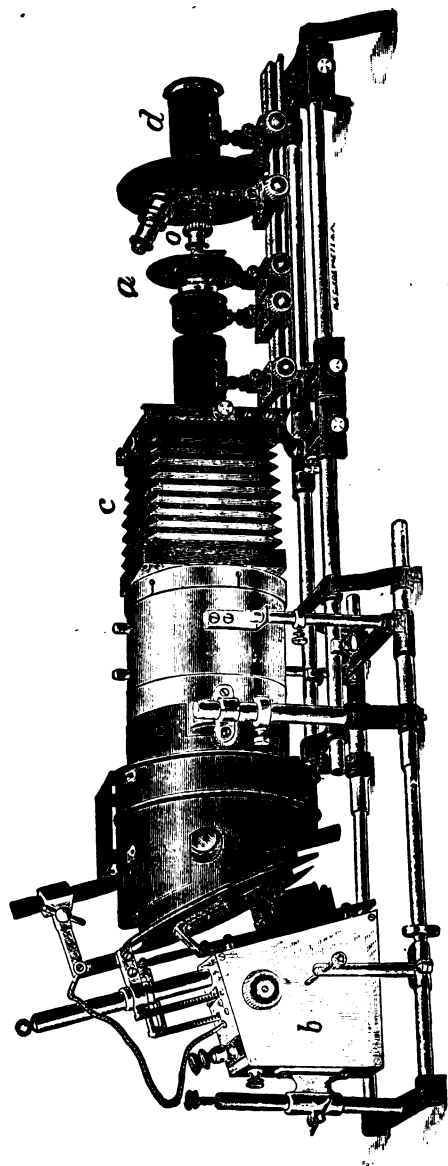


FIG. 95.—Colt's Criterion Lantern with Electric Lamp and Improved Microscope Attachment.

Where there are two lanterns in the laboratory, beautiful effects are secured by producing what is termed dissolving views. This is accomplished by placing the lanterns near each other, so that the beam of light thrown from each will cover the same portion of the screen; and, if the oxy-hydrogen jets are used, the key illustrated in Fig. 96 is so connected with the oxygen and hydrogen cylinders that the gases will pass through

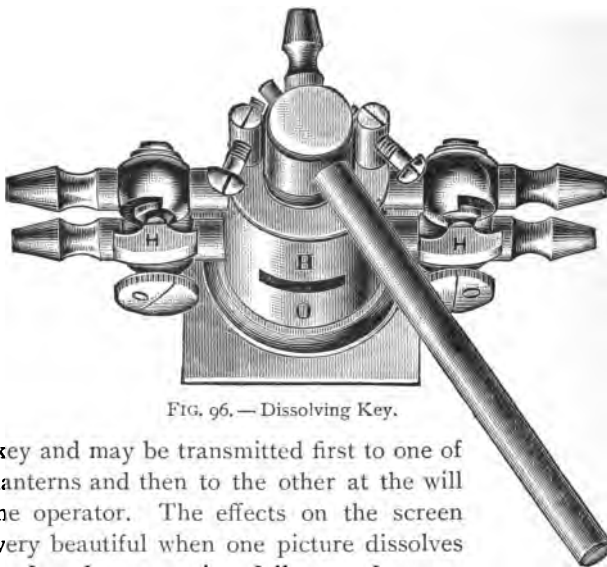


FIG. 96. — Dissolving Key.

the key and may be transmitted first to one of the lanterns and then to the other at the will of the operator. The effects on the screen are very beautiful when one picture dissolves off as the other grows into full strength.

The care of the lantern. — Like all other delicate apparatus the lantern is liable to serious damage unless carefully protected from dust and sudden changes of temperature. When the lamp is lighted the flame should be kept rather low until the condensers are gradually heated. For this purpose it is best to turn on the light ten or fifteen minutes before the pictures are projected on the screen, and then the light can be raised to its fullest intensity without so much danger of breaking the condensers and other glass lenses. An excellent protection against flying particles of highly heated lime and carbon is a piece of

clear mica interposed between the light and the lenses. The same care should be manifested in turning off the light to prevent the glasses from cooling too quick, because the unequal and rapid contraction will often cause them to break. When not in use the lantern should be carefully covered with a thick cloth to protect all parts of it from dust. Whenever it is necessary to clean any portion of the glass surface, the best material to use is Japanese paper, because it contains no particles of grit and is very soft and has considerable absorbent power for the moisture frequently condensed on the surfaces of the lenses. The rubbing, however, should be gentle, that no scratching may be done. *Rub the glass as little as possible*; it is much better to protect them from all particles of dust and sudden changes of temperature.

The light must be centred on the screen before the slide is put in the lanterns; so that a perfectly white light, free from all shadows and colors, will be formed. This is done by adjusting the lamp in three ways: vertically, laterally, and from or toward the condensers. This operation is very quickly accomplished when once learned. It is only necessary thereafter to adjust the front, projecting lenses as each slide may require.

The fact must be borne in mind, in determining the distance between the screen and the lantern, that the nearer the lantern is placed the smaller will be the picture; and, as a general rule, also, the greater the size of the picture the less will be the brightness of the disk of light on the screen.

CHAPTER XIV

APPARATUS AND METHODS IN THE STUDY OF BACTERIOLOGY

IN the study of bacteria it becomes necessary to adopt measures to determine the peculiar habits and methods of growth of these minute forms in order that the experimenter may arrive at conclusions concerning their life history. These microscopic forms must, therefore, be cultivated in the laboratory, and the steps of growth carefully noted and studied. In order to carry on this work to a successful conclusion the laboratory must be supplied with certain kinds of apparatus and chemicals not mentioned in the other portions of this work.

The special disease, or bacteria, must be developed by breeding experiments in flasks or test-tubes, and all the utensils and apparatus used in their cultivation must be carefully sterilized, so that no other organisms will enter the breeding vessels and confuse the observations on the special germ under examination. The intention of this chapter is to give in detail the steps to be taken, and the apparatus and chemicals required to conduct the culture experiments in a well-equipped laboratory.

The vessels may be sterilized by heating to a high degree (160° to 200° C.), until all living organisms which may be in the air of the vessel, or on its inner surfaces, are destroyed, and the vessel must then be kept closely stopped with cotton-wool which has also been sterilized. The nutrient fluids which are to be used in the culture experiments, or, in other words, which are to be the foods for the growing germs during their development, must also be treated with heat, so that all life in them will also be entirely destroyed before the bacilli to be studied are planted in them. This is accomplished in the following manner :—

The nutrient substance is boiled in a flask a short time each day for a period of several days, and the vessel must be closed with the cotton stopper. Fill the flask about one-half full, so that in the boiling the cotton will not get wet. Pull out the cotton about halfway during the boiling, and after finishing heating, push in the cotton again. A beaker with sterilized cotton is placed over the mouth of the flask, and the vessels are put aside over night. After boiling the first day certain bacteria are destroyed, but there are other spores which are able to stand higher temperatures; and after permitting the flask to stand a few hours after each boiling, these spores will have an opportunity to germinate, and a second boiling will also kill them. Hence the reason for successive heating on several different days. It has been found that after four or five days of treatment in this manner all life will be destroyed in the nutrient fluid, and it will then be in a condition for the transplanting of the special disease to be studied. By properly inserting the cotton-wool, there will be no danger of infection from the atmosphere, as long as the cotton remains in the neck of the flask. The greatest danger comes from the inner surfaces of the flask, which may have microscopic spores lodged thereon before the cotton was inserted, provided the flask was not properly sterilized. After the final boiling of the nutrient fluid, and after standing for some hours, its condition will indicate whether or no the substance is free from all organisms, and therefore suitable for the inoculation, or sowing, with the spores under treatment. When the final boiling is completed, the flask, plugged with sterilized cotton and covered with the beaker, is placed in the incubator and left there in a temperature of 32° to 38° C. for one to three weeks, and at the end of that period, if the nutrient fluid remains limpid, it may be used with safety and with the assurance that all bacilli are destroyed.

To sterilize vessels, such as flasks, beakers, and test-tubes, expose them for a while to a strong heat from a Bunsen burner. The heat must be applied to all surfaces thoroughly, and the

inner surfaces must be made specially hot. While in a heated condition the mouths of the vessels must be closed with the plugs of cotton one to two inches long, which have been also sterilized. Place these plugs in firmly but not too tight. When a number of vessels are to be sterilized this may be accomplished in the air-chamber, where a heat of 140° to 150° C. is sustained for a period of four to six hours. While in the hot condition the vessels are taken out, one at a time, and plugged with the sterilized cotton. They are then replaced in the air-chamber and the heat repeated for several hours.

The cotton is rendered sterile by pulling open the fibres and exposing them to a temperature of 150° C. in the air-chamber until a slight charring is evident. The heat is repeated for several successive days, so that all spores which may germinate between each heating will be destroyed. Care must be exercised to heat the cotton enough but not so far as to cause it to become brittle. It must retain its pliable condition, although some of the fibres have become slightly charred.

By observing the above precautions there need be no apprehension on the part of the experimenter that his experiments will be vitiated by badly sterilized vessels and cotton.

All metal instruments, such as knives, needles, etc., should be constructed to stand a strong heat without injury. And before use they should be subjected to the flame of a Bunsen burner.

The Apparatus used in Bacteriology

Hot-air and steam sterilizers. — There are several patterns in the market, and among the standard makes may be mentioned:

1. Pasteur's hot-air sterilizer.
2. Lautenschlaeger's hot-air sterilizer.
3. Bausch & Lomb's hot-air sterilizer.
4. Koch's steam sterilizer.
5. Arnold's steam sterilizer.
6. Autoclave, or steam digester, or pressure sterilizer. Several of the leading autoclaves are: —

- a.* Dr. Pfungst's autoclave.
- b.* Lautenschlaeger's autoclave.
- c.* Bausch & Lomb's autoclave.
- d.* Petri's autoclave.

These sterilizers may be classified as follows :—

- 1. Hot-air sterilizers.
- 2. Steam sterilizers.
- 3. Autoclaves or pressure sterilizers.

Hot-air sterilizers. — This apparatus, Figs. 97 and 98, consists of a metal box containing shelves and covered with suitable material to prevent heat radiation. The heat is applied by means of a Bunsen burner at the base of the apparatus. Attached to



FIG. 97. — Hot-air Sterilizer. (B. & L.)

this sterilizer are also thermometers and thermo-regulators, so that the degree of heat may be under control and a constant temperature may be retained as long as desired. The hot air circulates not only over the bottom of the apparatus but also up

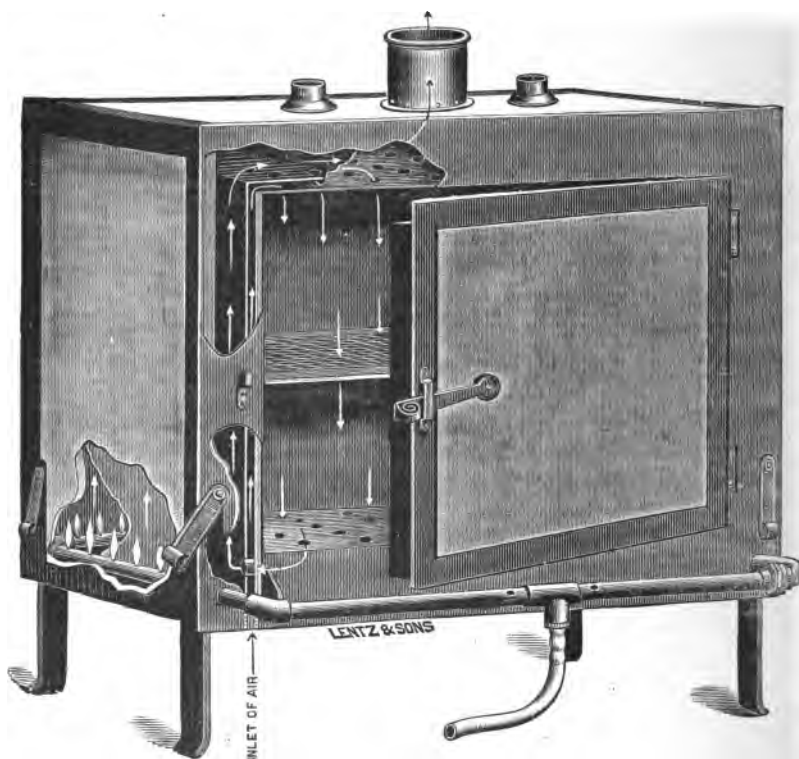


FIG. 98. — Hot-air Sterilizer, Lautenschlaeger's Pattern.

the sides and over the top, so that the degree of temperature is uniform over the entire instrument. The sterilizer has double sides in order that the air may be thus circulated. The arrows in the illustration show which way the gas goes when the heat is applied at the bottom of the apparatus.

Steam sterilizers.— These instruments are generally used to sterilize nutrient media, and for filtering fluids, which are difficult to filter except when hot. The apparatus is made of copper with a sterilizing chamber so arranged that the articles placed in it are subjected to a current of steam. This instrument is also provided with thermometers, and the whole is covered with



FIG. 99. — Arnold's Steam Sterilizer.

a non-conducting substance to prevent heat radiation. The Arnold form of the steam sterilizer (Figs. 99, 100, and 101) is operated by placing water in the pan or lower reservoir, where it passes by degrees into the lowest compartment through small openings to be heated by the burner and driven into steam. The stream rises through a central shaft to the sterilizing chamber, where the vessels are located. The action is almost instantaneous because the water film in the lowest compartment is so thin that

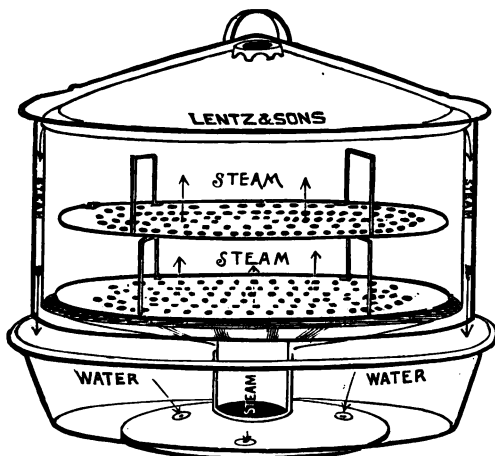


FIG. 100. — Arnold's Steam Sterilizer. Section.

the heat causes it to boil at once and the steam rises rapidly into the chamber around the vessels to be sterilized. There is thus around the vessels a constant temperature of 100°C . The excess of steam escapes through the cover or door.

Autoclave, or pressure sterilizer.

— Some spores resist the action of heat more strenuously than others, and it becomes necessary to apply a higher degree of heat than can be secured with the usual form of hot-air or steam sterilizers, at the ordinary atmospheric pressure; so the autoclave (Fig. 102) has been devised to give steam under high pressure with temperature raised to 135°C . Thirty pounds' pressure to the square inch can be obtained by means of this apparatus, which has been found to be equivalent to 134.6°C . A pressure-gauge is attached to the autoclave to measure this pressure, and an escape-valve is also connected so that the

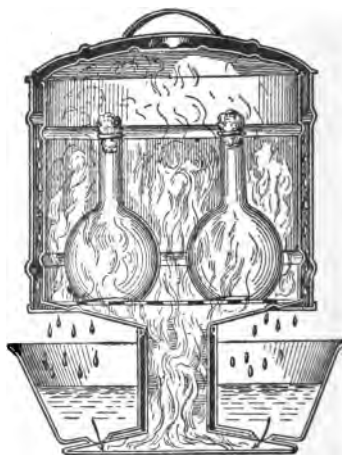


FIG. 101. — Arnold's Steam Sterilizer. Section.

action of the steam may be under control. A thermometer gives the temperature, and an air-valve is placed on the ap-



FIG. 102. — Autoclave.

paratus, which must be kept opened until the air is all driven out by the steam, which is indicated by the blowing out of the

steam. This autoclave can be also used as an ordinary steam sterilizer at the normal temperature if desired; so that if the laboratory possesses an autoclave the steam sterilizers may



FIG. 103.—Laboratory Incubator. (B. & L.)

be omitted from the list of apparatus when the equipment is purchased. The heat is applied by means of a ring Bunsen burner.

The autoclave costs more than either the hot-air or steam

sterilizer, but the laboratory in which work is carried on in experimenting with plant and animal diseases cannot well afford to do without it.

Incubators.— These instruments (Fig. 103) are absolutely essential in the laboratory where culture experiments are conducted.

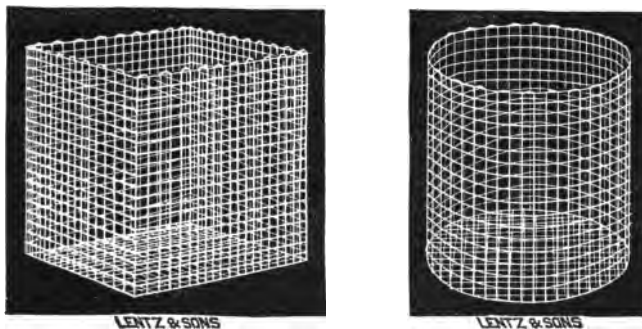


FIG. 104.— Wire Baskets.

The incubator is used to grow the bacteria in a temperature which is under control by a thermo-regulator. The illustration shows the usual form to be found in the large laboratories. There are two doors, one of plate glass and one solid; both are close fitting, so that they are practically airtight. The heat is communicated by means of a Bunsen burner in the lower compartment, and this burner is of the double Koch safety pattern. The incubator is constructed to hold water between the double walls, and there is also a hot-air chamber. The heat being under perfect control, the growing cultures can be developed under known and fixed temperatures.

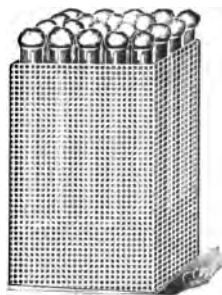


FIG. 105.— Wire Basket containing Culture Tubes.

The shelves are used to hold wire baskets in which the test-tubes containing the cultures are stacked. Figure 104 shows two forms of this basket.

Gas pressure regulators.— In the use of incubators and sterilizers it is of the utmost importance that the heat should be regulated, and this must be accomplished by an automatic control of the flow of gas to the burners. The gas must first pass through a pressure regulator and then through a thermo-regulator before it reaches the burner. There are several instruments on the market for securing these ends. The description of Reichert's given below will explain the general principles controlling them all.

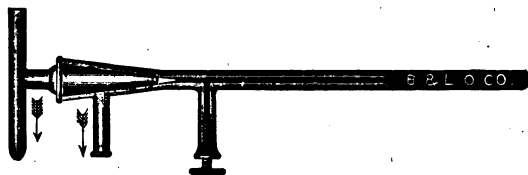


FIG. 106. — Reichert's Thermo-regulator.

Reichert's thermo-regulator (Fig. 106).— There are two portions of this instrument, a hollow T-shaped piece, in which one end of the horizontal section is open and the other end is closed, a tube containing mercury and two horizontal orifices. At the lower end of the T-tube are two small holes, one at the point and the other on one side. The instrument is placed in the roof of the incubator so that the bulb is in contact with the water or extends in the interior of the chamber, and the gas is passed through a rubber to the open end and out into another rubber tube leading to the burner. When the desired temperature is reached the mercury is forced up the tube by means of the screw until the orifice in the T-tube is closed, excepting the small orifice on the side, when the flame will lower and the heating will be

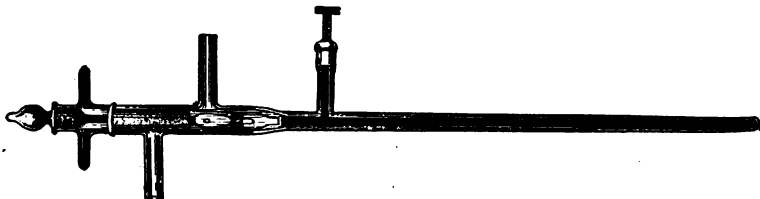


FIG. 107. — Novey's Thermo-regulator.

diminished. The mercury will then recede and the flow of gas will again increase, and in this way the temperature will be controlled automatically.

Dr. F. G. Novey has recommended a change in Reichert's regulator which is designed to prevent the passage of more gas through the minimum opening than is required to maintain the desired temperature (Fig. 107).

Nutrient Media and their Preparation

Nutrient media are the chemicals used in the laboratory devoted to bacteriological research, for the food of the bacteria during the process of culture. In the study of diseases of plants or animals it becomes necessary to grow the bacteria under known conditions in order that the life-history of the germ may be well understood; and to do this work satisfactorily the germ must be developed in a medium which is a food for the spores when they begin to germinate, and which at the same time is sufficiently transparent so that the experimenter may observe the different stages of growth.

There are generally three kinds of these nutrient media, viz. :

1. Nutrient bouillon.
2. Nutrient agar-agar.
3. Nutrient gelatin.

Several other substances are used in the laboratory as food for the bacilli, such as slices of potatoes, carrots, turnips, blood serum, etc., but the media generally used are the three mentioned above. In the preparation of these nutrient substances the following steps must be taken.

Nutrient Bouillon. — Add 1000 cc. of water to 500 grammes of finely chopped, or ground, lean beef, freed from fat. Place the vessel containing this beef in the water-bath and warm gently to a temperature of 55° to 60° C. for the period of an hour, with frequent stirring. If the temperature goes above 60° C. the albuminous substances will coagulate, which will somewhat interfere with the final clearing of the fluid. After heating for an hour, and when the soluble matter of the beef has been dis-

solved, the mass is strained through a linen cloth. The filtrate is made up to 1000 cc. by the addition of water. Add to this fluid, which is a deep red color, five grammes of salt and ten grammes of a 1 per cent Witte's peptone, and warm until solution is complete, but taking care not to let the temperature go below 60° C. Render the solution slightly alkaline by the addition of a saturated solution of sodium carbonate; test carefully with litmus-paper¹ until the red slightly turns blue. Boil for about a half-hour and filter. Cool and distribute in Erlenmeyer flasks or test-tubes and plug with cotton. The usual quantity for each flask is 250 cc. Sterilize for fifteen minutes in the steam sterilizer each day for three days, before inoculating with the germ submitted for investigation.

Nutrient Agar-agar. — Agar-agar is a seaweed found on the coast of Japan, and is sometimes sold under the name of Bengal isinglass because this seaweed is also found near Singapore. When dissolved in water it yields a thick, odorless jelly.

In the preparation of this nutrient, bouillon is used as the base. The agar-agar is added for the purpose of solidifying the mass in the form of a jelly. Add to the liter of bouillon 20 grammes of agar-agar which has been finely cut up. Place the vessel in the autoclave, or steam sterilizer, and heat until the agar-agar is thoroughly dissolved. This will generally take from one-half to one hour. The vessel is then placed in the water-bath at a temperature of 60° C. until the solid particles settle to the bottom, when the fluid is filtered through absorbent cotton. The filtering of this nutrient agar-agar is a very slow and tedious work. Dr. M. P. Ravenel gives the following method for preparing and filtering this nutrient, which he claims to be superior to any other method both in the time saved in filtering and in the character of the fluid obtained :² —

¹ G. W. Fuller titrates with phenolphthalein in place of litmus, as an indicator in adjusting the reaction of the media. Comparative experiments on alkaline media showed that water bacteria thrive best on those which contain 15 to 20 cc. normal alkali per liter. (*Jour. Amer. Pub. Health Ass.* X, 1895.)

² *Jour. App. Mic.*, Vol. I, p. 106.

"To make one liter of agar-agar, take

A. Dried peptone (1%)	10 grammes
Common salt (0.5%)	5 grammes
Liebig extract (0.5%)	5 grammes
Water	500 cc.

"Boil for three minutes and neutralize.

B. Agar-agar (1.2%)	12 grammes
Water	500 cc.

"Chop the agar-agar and put into autoclave. Run the autoclave up to two atmospheres of pressure, giving 121.4° C. of heat. As soon as this pressure is reached, turn out the flame and allow the autoclave to cool until below 100° C. before opening. The two solutions A and B are then mixed, cooled to 60° C., the whites of two eggs beaten in 50 cc. of water added and well stirred in, and the whole then boiled and filtered through paper.

"The whole process requires only an hour and a quarter to an hour and a half, and the result is a most excellent jelly. Instead of white of egg, blood serum may be used, which seems to add also to the nutritive value of the medium. Agar made with meat extract will often form a precipitate during the sterilization, which is objectionable if one wishes to use it in the making of Esmarch's roll-tubes.

"To make an absolutely and permanently clear agar, fresh meat should be used as follows:—

"To make one liter, take

A. Chopped meat	500 grammes
Water	500 cc.

"Mix and place in cool place over night, then strain through towel.

B. Agar-agar (1.2%)	12 grammes
Water	500 cc.

"Put in autoclave, run up to two atmospheres of pressure, put out flame, and allow to cool until below 100° C. before opening. Let the solution of agar cool still further to about 75° C.,

and then mix A and B, add (1%) 10 grammes dried peptone and (0.5%) 5 grammes of common salt, bring to a boil for about three minutes, neutralize and filter. The product is an absolutely clear jelly which never forms a precipitate. The whole process, with the exception of the time the meat is steeping, requires only about one hour and a half. In both of the above methods the filtration is very quick— from ten to twelve minutes for the liter. I never use a hot-water funnel, but wet the filter-paper with boiling water immediately before pouring in the agar. In the process with fresh meat the clarification is effected by the coagulation of the albumen in the meat water, hence solution B must not be added to A until cool enough to avoid coagulation. In general the fresh meat is to be recommended, and the process is easier than with the meat extract, though the latter has the advantage of cheapness and convenience, since the meat extract can always be kept on hand, and the time lost in soaking the fresh meat is saved."

Nutrient gelatin.—The bouillon is also the base of this nutrient. In its preparation 1000 cc. of bouillon are taken, and to the solution are added 5 grammes of common salt, 100 grammes of gelatin, and 10 grammes of Witte's peptone (1%). Heat is applied in the water-bath until the gelatin is dissolved. The fluid is then rendered slightly alkaline by the careful addition of a saturated solution of carbonate of soda. The vessel is again placed on the water-bath and the water in the bath boiled for one hour. The albuminoids in the nutrient separate in flakes, when the fluid is filtered. The filtrate must have the following properties:—

1. Solidify on cooling.
2. Be perfectly clear and remain so after heating in the sterilizer.
3. Show slight alkaline action.

The nutrient gelatin and nutrient agar-agar have different melting-points, and this fact must be borne in mind when using them for culture experiments. The first melts at the temperature of the body, while the latter requires a temperature as high

as boiling-point. Some bacteria require higher temperatures for their growth than do others, and in such cases the nutrient agar-agar must be used.

Potato culture method.—Select a sound potato, and cut off the ends and core with an apple-corer, making the sections about 25 mm. in diameter. Make an oblique section across the centre of the core, thus dividing it into two pieces. Place in running water for a time to prevent discoloration, and then submit to the action of a dilute alkali solution in order to render the pieces uniform in neutral or slightly alkaline reaction. Now put these sections of the potato in test-tubes with the oblique faces upward, sterilize an hour each day for several days in a steam sterilizer, and then inoculate with a sterilized knife containing the material for study.

Blood serum.—Wash the vessels which are intended to receive the blood, first with sublimate, then with alcohol, and finally with ether. The latter is allowed to evaporate. The place on the skin of the animal (sheep, calf, or horse) is washed with sublimate, and an incision is made with a sharp sterilized scalpel. The first outflow of the blood is rejected, and then the quantity desired is received in the vessels as above prepared, and the glass stoppers, greased with vaseline, are replaced, and the vessels are quickly transferred to ice, where they remain for twenty-four to thirty-six hours. After the clot separates, the clear serum is drawn off into sterilized test-tubes, which are closed with cotton plugs. Now sterilize with a temperature of 80° to 90° C. for one hour, and repeat the operation for six successive days. To solidify the serum raise the temperature to 100° C., and the tubes are then ready for inoculation. The color of the serum should be pale straw, and transparent. In addition to this, serum of one-third its volume of nutrient bouillon containing 10 per cent of glucose gives a medium for the cultivation of diphtheria bacillus.

Inoculation.—This is the term employed to designate that operation adopted by the experimenter in inserting the bacteria in the nutrient media for the purpose of causing the germs to

grow and develop. There are seven methods usually in practice among bacteriologists for the accomplishment of this operation :

1. Tube culture.
2. Dilution method (after Nägeli and Lister).
3. Fractional culture (employed by Koch).
4. Hanging-drop culture (after Koch).
5. Plate culture (after Koch).
6. Esmarch's roll culture.
7. Anaërobic culture (bacteria excluded from air).

Tube culture. — The test-tubes, which are employed in this method, are carefully cleaned, plugged with cotton, and sterilized. In each tube is placed 12 cc. agar-agar nutrient, or nutrient gelatin, and care exercised so that the sides of the tubes are not smeared with the fluid. The tubes are then placed in the wire baskets and transferred to the steam sterilizer for fifteen minutes each day for three days, and during the intervals they are kept in the incubator at the temperature for spore germination. If at the close of the third day the nutrient fluids come from the incubator clear and without spots, it can be assumed that all life has been destroyed in the tubes. The end of the test-tube, containing the cotton plug, is passed through the flame of a Bunsen burner until the latter ignites, when the flame is put out. This action is for the purpose of destroying all bacteria that may have lodged in the dust settling from the air. Now, with a fine-pointed pipette, which has been previously carefully sterilized, transfer to the nutrient in the tube some of the preparation containing the bacteria which is to be subjected to examination. This inoculation is accomplished in the following manner : Raise the cotton plug slightly and insert the pipette until it just touches the nutrient agar, and then press it slightly below the surface of the nutrient until the bacteria in the pipette find lodgement in the fluid. For purposes of satisfactory examination, it will be well to place the bacteria near the surface, and as the colonies grow the results can be more readily determined than if the inoculation had been made near the centre of the nutrient agar. If the test-tubes were somewhat inclined after the liquid nutrient

was placed in, the solidification would form an oblong surface and thus expose more area for inoculation.

Perform this work of inoculation in as short a time as possible so as to avoid every chance of contamination from the germs in the air. Place the inoculated test-tubes in the incubator and keep the temperature at 35° C. to 37° C. If at the end of a day or so the agar shows a turbid condition, or spots of color at places near the surface, the experimenter may rest satisfied that his work is progressing well.

Keep careful notes of the following items : —

1. Source of bacteria under study.
2. Character of nutrient used.
3. The effects on the nutrient.
4. Time that growth began.
5. Temperature sustained in the incubator.
6. The miscellaneous appearance of the colonies.
7. Make drawings of all stages of development.

Dilution method. — The use of this method of culture is to separate the bacteria into individuals, or at least into small, detached colonies containing a small number of individuals of the same species. This work is done for the purpose of facilitating the study of the special disease, and to determine how many species of bacteria are involved. This method was devised by Nägeli and Lister, and consists in diluting with sterilized distilled water, or other indifferent fluid, the culture fluid containing the bacteria, and then, by means of the pipette, inoculating other tubes which have sterilized nutrient agar with a fraction of a drop from the diluted mass of bacteria. Or a drop is drawn from the diluted mass and the pipette containing the drop is dipped in a fresh supply of sterilized distilled water, or well-boiled saline solution (0.6 per cent), and the liquid is drawn up into the pipette so that the dilution of the drop is carried still further, one thousand fold or more, and from this dilution other tubes are inoculated with the smallest fraction. These freshly inoculated tubes are now placed in the incubator and the cultures developed.

After the expiration of twenty-four hours an examination of the tubes is made, and it will be noted that some have clearly defined spots showing bacteria growths of probably one species, while other tubes have their nutrient agar unchanged, showing no inoculation from the portion of the diluted fluid transmitted to them from the pipette. Of course it will be readily seen that this dilution may be carried to great degree. Instead of using the pipette to accomplish the dilution, a drop of the first diluted mass may be placed in a liter of the sterilized distilled water and a dilution of 1 : 1,000,000 or even more be secured.

Fractional culture. — This is Koch's method. The culture is based on the fact that one species of bacteria will grow readily under certain conditions of temperature of the nutrient fluid, while another species will not thrive so well; the first, therefore, will ultimately crowd out the latter and take possession of the field. The steps of procedure are as follows: —

Inoculate a tube with sterilized nutrient fluid, in the manner described under tube culture, and place it in the incubator. After the colony is under good process of growth, a small portion is extracted by means of the sterilized pipette and transferred to a fresh tube of sterilized nutrient fluid, and this is also placed in the incubator, where a constant temperature is maintained of 35° C., and after the growth begins, a third tube is inoculated from this and permitted to germinate as before, and a fourth tube is inoculated from the third, and so on until the reduction is carried so far as to insure the separation of the species; and it is claimed that in this manner it is possible to obtain pure cultures of individuals of the same species.

Hanging-drop culture. — This was also devised by Koch, and is an excellent method for examining the growth of bacteria in all phases under the microscope, which is rather difficult of accomplishment with the other methods of culture without extracting some of the culture and mounting it on a glass slip.

Make a high ring with glass or rubber (Fig. 108) on a slip by cementing to the slip two or more glass rings sold by any of the dealers in microscopical material. After the cement well hardens,

wash in a solution of corrosive sublimate and place in the hot-air sterilizer. Clean in the same manner a cover-glass and sterilize; place in the centre of this glass a drop of sterilized nutrient fluid. Inoculate in the usual way and put the cover-glass on the ring, around the edges of which has been smeared sterilized vaseline. Transfer to the incubator in a temperature of 35°C. , and after the growth has begun, the slide can be placed on the



FIG. 108. — Hanging-drop Culture.

stage of the microscope and the development of the bacteria examined at pleasure without disturbing the colony.

Plate culture. — This is also a method for isolating bacteria and the separation of species into distinct colonies.

A tube of sterile nutrient fluid is warmed until the fluid becomes liquid, when it is inoculated with a trace of bacterial mixture by means of the sterilized pipette, and the mass is well shaken so that the bacteria are well distributed throughout the nutrient agar or gelatin. This is now quickly transferred to the bottom of a Petri dish and spread out over the surface. The Petri dish, resting on a glass plate, is at once covered by another slightly larger dish, and over these dishes is placed a bell-glass jar, around the edges of which is smeared vaseline, so that the contact with the glass plate will make an air-tight connection. In the bell-glass jar is also placed dampened blotting-paper, in order that the air may be kept in a moist condition. Of course, in all of these experiments it must be understood that all dishes and utensils used are well sterilized before the inoculation is performed. The nivellating apparatus illustrated in Fig. 109 will enable the experimenter to level the plate culture, and thus preserve a uniform depth in the nutrient fluid.

If the nutrient fluid is gelatin, the temperature of the incubator in which the Petri dishes have been placed must be kept at a temperature near 20° or 22°C. to insure the solidity of the

gelatin. If agar-agar is used in the place of gelatin, the temperature can be raised to 35°C . Experiments have proven that some bacteria thrive well at the temperature required for gelatin, while they would be destroyed or retarded in growth if placed in agar with a temperature at 35°C . Other forms require higher temperatures to germinate and produce the best results, so that agar-agar nutrient must be the fluid used for the experiment.

If a still further differentiation of the bacteria is desired, a

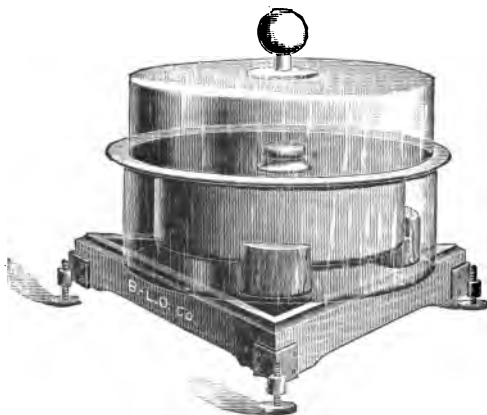


FIG. 109.—Nivellating Apparatus.

small portion of the nutrient fluid may be taken from the Petri dish after growth begins and a new plate culture developed. In this manner the species may be separated into colonies for detailed study.

Esmarch's roll culture.—This method was extensively

used before the introduction of the Petri dish method, but it has now been almost entirely superseded by the latter. Like Koch's plate culture, the prime object of the roll culture is for the purpose of separating the bacteria into colonies of distinct species, and the *modus operandi* consists in liquefying the fluid nutrient gelatin in a large tube, inoculating, and revolving the tube inclined on a block of ice until the gelatin solidifies on the sides of the tube. Care must be taken not to permit the gelatin to come in contact with the cotton plug. It is best to use gelatin with this method, because the agar does not adhere so well to the sides of the glass tube.

Anaërobic culture.—Some bacteria do not grow freely in oxygen, and in the study of such forms it becomes necessary to

substitute a gas for the oxygen or air, or to cultivate the bacilli in bouillon covered with a substance impervious to air. There are several devices proposed by workers in bacteriology for accomplishing this exclusion of the oxygen of the air. One of the most simple and effective is that suggested by Dr. Theodore Kasparec of Vienna:¹—

“In order to be able to cultivate tetanus bacilli in bouillon in a simple manner, I prepared the cultures in ordinary flasks and

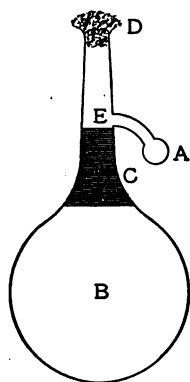


FIG. 110.

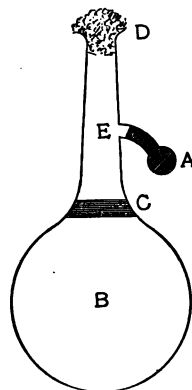


FIG. 111.

Kasparec's Anaerobic Culture Apparatus.

selected the paraffin method, as recommended by Kitasato, for excluding the air. As pouring the liquid paraffin upon the inoculated bouillon did not appear practical enough, on account of the danger of foreign germs entering while pouring, the smearing of the neck of the glass with paraffin, as well as the influence of the heat of the melted paraffin, I had a special flask made for the purpose, which had proved very reliable in keeping the culture air-tight.

“The flask intended for the purpose may be a spherical one, may be of any desired size, with a rather long neck tapering toward the top. A small tube terminating in a bulb is blown

¹ *Central-Blatt f. Bakt.*, Bd. XX, p. 536, and *Jour. App. Mic.*, Vol. I, p. 34.

into the side of the neck of the flask about one centimeter from *C* (Fig. 110). The flask is first filled with bouillon almost to the neck, and about three cubic centimeters of liquid paraffin are then added, after which the whole is sterilized in the steam sterilizer. The heat expands the bouillon, causing the paraffin to rise in the neck of the flask and overflow into the side neck and small bulb *A* (Fig. 111), so that after sterilization there is only a very thin film of paraffin remaining on the top of the culture medium at *C*.

"During the heating a large portion of the air absorbed by the bouillon is driven out, and its reabsorption while the flask is cooling is prevented by the thin film of paraffin. After cooling, the paraffin hardens into a solid coating, which can be readily pierced when it is desired to inoculate the bouillon. After inoculation the hardened paraffin in the small bulb is liquefied by heating slightly, when it may be poured upon the film already formed above the bouillon by slightly inclining the flask. Upon hardening this additional paraffin constitutes an almost perfectly

air-tight layer, which becomes even more effective by being pressed into the tapering neck when the culture is heated in the incubator. When pouring out the cultures after growth has taken place, the paraffin is again led into the lateral bulb, after warming the vessel, by inclining the flask."¹



FIG. 112.—Novy's Culture Apparatus for Petri Dishes.

Another simple device, suggested by Turro in *Cent. f. Bac.*, I, 31, 175, is to use a Petri dish with a glass circular plate resting on glass supports extending from the bottom to within a half-inch of the top of the

dish. The gelatin culture medium is spread over the circular glass top and inoculated, and then inverted to its position on

¹ For a full discussion of the methods of cultivating anaërobic bacteria, see *Four. App. Mic.*, Vol. V, p. 1800.

the glass supports in the Petri dish. Before this top is placed on, however, there is poured into the dish a quantity of pyrogallic acid and NaOH, and then the top is sealed down with melted paraffin, which will prevent the entrance of air. The pyrogallic acid will soon absorb the oxygen remaining in the dish, and it will then be possible for the bacteria to grow free from the oxygen.

Novy has devised an apparatus of tube and plate cultures by the gas or pyrogallate methods (Figs. 112 and 113), which possesses some advantages worthy of consideration. This instrument, as shown in Fig. 119, is a glass vessel made in two sections, so that Petri dishes as well as tubes can be placed in it. The top section is clamped on and made air-tight, and the hydrogen gas passed through until the air is driven out. In the pyrogallate method, 3 to 5 grams of pyrogallic acid are placed in the bottom of the apparatus in a dish and the Petri dishes placed on top of it, with a narrow strip of glass intervening. The apparatus is now closed, excepting a narrow slit, through which is introduced as quickly as possible 25 cc. of concentrated potash solution (1:4).



FIG. 114.—Wolffhuegel's Counting Apparatus.



FIG. 113.—Novy's Culture Apparatus for Tubes.

The top is then securely fastened down. (See *Jour. App. Mic.*, Vol. II, p. 267.)

Wolffhuegel's counting apparatus.

—It now becomes necessary, in the culture of bacteria, to know how many individuals belong to the colony, and in order to do so with any degree of accuracy a counting apparatus must be used. The above is one of the best (Fig. 114), and is well known among microscopists. The rulings on the plate are 1 centimeter square, and there are

diagonal rows of these squares ruled to 0.3 centimeter squares. There are two background plates, one white and the other black, so as to facilitate the reading of the measures on the instrument. The culture plate or the Petri dish is placed on this instrument, and the individual bacteria are counted which cover one of the graduated squares and an estimate made from this for the entire colony.

CHAPTER XV

BLEACHING, DECALCIFICATION, INJECTION, MACERATION

Bleaching. — Some of the objects subjected to microscopical examination are so opaque with pigments and other matters, it becomes necessary to bleach or clarify them with some chemical which will act on the pigment and dissolve it out of the specimen. The chemicals generally suitable for this purpose are so active in their attack on the subject to be eliminated that, unless great care is exercised, the delicate tissues will be seriously injured. Of course it will be readily understood that in the case of those experiments where there are soft cell contents, or the fine markings on the delicate cell walls, then the strong alkalis and acids used in bleaching must not be employed.

It becomes necessary also to bleach when specimens have been overstained in such stains as chloride of gold and nitrate of silver or fixed in osmic acid solutions. When chlorid of gold, which has been used in staining nerve tissues, has overstained the section, the extra color may be extracted by using cyanide of potassium. This chemical may also be used in treating sections overstained with nitrate of silver. The cyanide of potassium should be dissolved in water at the rate of 1 : 10.

Insects can be bleached in a solution of peroxid of hydrogen, so that the respiratory organs may be clearly and satisfactorily examined under the microscope.

A weak water solution of potassium hydroxid is a bleaching agent which acts on protoplasm and transforms it into a clear mass. It also acts on the cell walls. Hanstein used it in his experiments on merismotic tissue and germ development. After treatment in the potassium hydroxid, wash and soak in glycerin diluted in water or alcohol, when the specimen will become



clear and so transparent that the cell walls almost disappear, but by treating with weak solution of alum the walls reappear. Resins and fat masses will yield to the action of this bleaching agent by soaking for a short time in the moderately strong solution of the chemical and then washing with strong alcohol. If the tissue shrinks, because of the too rapid action of the gums and the strong effects of the alcohol, the cells can be made to retain their normal shapes by imperfectly washing with the alcohol and the repeated application of water. Transfer to water containing a little hydrochloric acid.

Bleaching animals and sections fixed with osmic acid solutions (Dr. D. Carazzi, *Zool. Anzeig.*, XVII, p. 135, 1894). Peroxid of sodium is used (Na_2O_2). When dissolved in water the yellow powder becomes alkaline and forms caustic soda. Mixed with tartaric acid or acetic acid the soda combines with the acid. A 10 per cent solution of the acid is put in a vessel, and a small quantity of the peroxid of soda added; then 70 per cent alcohol is slowly dropped on the surface of the solution. Place the object in the alcohol thus added to the peroxid of soda, and as the oxygen escapes from the water and rises in the alcohol it is slowly dissolved and the specimen is bleached.

Marsh's method for bleaching.— This consists in the application of free chlorin to the specimen. Two wide-mouth bottles, *A* and *B* (Fig. 115), are provided with securely fitting corks. In *A* is placed potassium chlorate and hydrochloric acid, and in *B* is water with the specimen to be bleached. Connect the two bottles with a glass tube *C*, so that the end which enters the bottle *A* will go just below the under surface of the cork, and the other end in *B* must extend to the bottom of the bottle. The chlorin gas evolved by the action of the hydrochloric acid on the potassium chlorate will pass over into the bottle *B* and bleach the specimens, and the extra gas will escape through a notch in the cork at *D*. Wash in running water after bleaching until all of the chlorin is taken out of the specimens.

Mayer's chlorin method differs from Marsh's in the way the crystals of chlorate of potash are used. The vessel containing

the chlorate of potash and the hydrochloric acid also contains the specimens to be bleached. As soon as the chlorin gas begins to evolve, a few cubic centimeters of 50 to 70 per cent alcohol are added, and the specimens, previously soaked in 90 per cent alcohol, are placed in the alcohol floating on the chlorate of potash solution. They soon sink to the bottom,

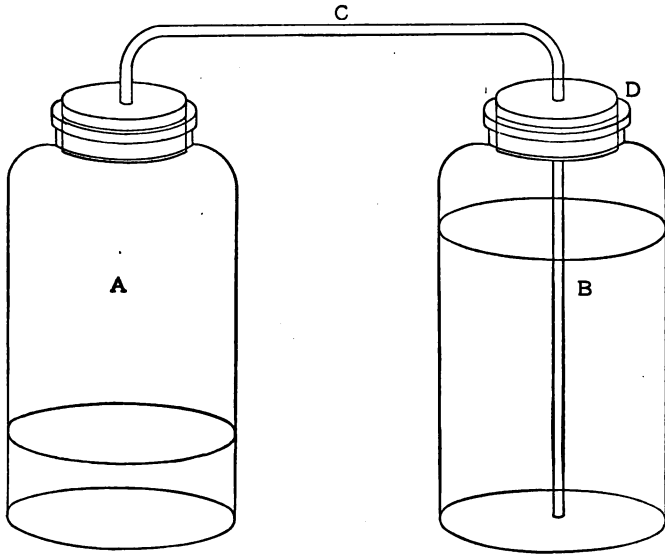


FIG. 115.— Marsh's Apparatus for Bleaching.

and the bleaching will be completed in a few hours. This method will correct the staining due to osmic acid, and the chlorin gas will also eliminate natural colors in the sections. The action is aided by placing the apparatus in the water-bath under a gentle heat. After bleaching remove the specimens to pure alcohol.

Decalcification.— In order to secure satisfactory sections of bones, teeth, and other specimens containing calcium, it becomes necessary first to extract all the lime salts. The agents generally used for this purpose are hydrochloric and nitric acids.

The acids must be in a diluted form, and since in the case of the hydrochloric acid swellings of the tissue result, chromic acid or alcohol must be combined with it to prevent in a measure this trouble. A solution of common salt, 10 to 15 per cent, added to a 3 per cent solution of hydrochloric acid will also prevent the swelling. Nitric acid does not produce this trouble.

Before decalcification the specimen must be well fixed in alcohol or in picric acid, or in Tellyesmiczky's acetic bichromic solution, viz. :—

Bichromate	.	.	.	.	.	.	.	.	3 grammes
Glacial acetic acid	.	.	.	.	.	.	.	.	5 cc.
Water	.	.	.	.	.	.	.	.	100 cc.

The specimens must be treated in large quantities of the decalcifying solution with frequent changing to fresh quantities. After the decalcification is completed wash in running water, or soak for several days. Now transfer to several strengths of alcohol to harden and dehydrate.

Haug's decalcifying solution.—This is one of the most rapid decalcifying agents known to microscopists. The addition of the phloroglucin is to reduce the injurious effects of the strong nitric acid. Bones placed in this solution will become decalcified in a few hours, but it is best to watch carefully the action of the solution because of its very rapid work. Wash several days in running water.

Phloroglucin	.	.	.	.	.	.	.	.	1 gramme
Nitric acid (pure)	.	.	.	.	.	.	.	.	10 cc.

Warm slowly and add 100 cc. of water and 10 cc. nitric acid. After washing the tissues pass them through 70 per cent and 95 per cent alcohol.

Dr. E. Rousseau has devised the following method for decalcifying :—

“The objects to be decalcified, which should not be too large, are imbedded in celloidin in the ordinary way, *i.e.* after fixation, are dehydrated in alcohol, and then, after having been immersed in a mixture of equal parts of ether and alcohol, are impreg-

nated with celloidin in solutions of increasing strength (4 per cent, 8 per cent, 12 per cent). When the objects are sufficiently saturated with celloidin, the latter is hardened by slow evaporation, by alcohol, or by chloroform. The celloidin blocks containing the objects are next immersed in a mixture of alcohol at 90° and nitric acid, the proportion of the latter being regulated by the amount of calcareous matter to be got rid of. The author uses 10 to 50 parts of nitric acid to 100 of alcohol. The decalcifying fluid should be renewed from time to time, and when decalcification is complete, the excess of acid is removed by washing in water and immersion in 90 per cent alcohol, frequently changed for several days. When all the acid has been removed the celloidin block may be sectioned. Now it may happen that, owing to extensive decalcification, there will be large gaps in the objects which would be a source of considerable inconvenience in making and manipulating the sections. The difficulty is, however, easily overcome by filling up the holes with 4 per cent celloidin, and allowing this to set by evaporation. This method possesses many advantages — there is no deformity of the animals or tissues, which are observed *in situ*, and it is easy and rapid." (*Jour. Roy. Mic. Soc.*, p. 375, 1898.)

Injection. — In normal histology it is a common practice to fill the vascular system with coloring matter when it is desired to study the arterioles and capillaries of animals. The method of injecting the coloring matter into the arteries and veins of the animal is difficult, and success can only be secured by long practice. The results, however, are exceedingly beautiful, and, in some cases, the injection is necessary in order to show the relation of the capillaries to the other elements of the organ under examination. The student should not become discouraged if his first attempts are not successful, but he must have patience and persevere if proficiency in the work is to be attained.

The coloring matter is held in suspension in either glycerin or gelatin, and the character of the mass depends on the kind of color to be injected. The glycerin and gelatin are termed the

vehicles, and the combination of the vehicle with the color is called the *injection mass*. Glycerin is preferable to gelatin because of its convenience and the good results secured, and because the mass can be used cold. Glycerin masses, however, have defects, as stated by Lee in his "Vade-Mecum," "for injection of fresh specimens — that is, those in which rigor mortis has not set in; they stimulate the contraction of the arteries. In these cases it may be advisable to use nitrite of amyl as a vasodilator. The animal may be anæsthetized with a mixture of ether and nitrite of amyl, and finally killed with pure nitrite."

To inject with gelatin some difficulty is experienced because of the necessity of keeping the mass and the instruments and the object in a warm condition. There are two gelatin masses which are generally used for injecting, viz.: Fol's carmine and gelatin mass, and Thiersch's Prussian blue gelatin mass.

Good photographic gelatin is soaked for several hours in a small quantity of water, the water is then poured off and the mass warmed over the water-bath until the gelatin melts. Carmine is added to strong ammonia, diluted with three parts of water until saturation, and the solution is poured into the gelatin while stirring. The correct proportions are:—

Gelatin	.	.	.	.	.	.	.	.	1 kilogram
Water	.	.	.	.	.	.	.	.	small quantity
Carmine-ammonia	.	.	.	.	.	.	.	.	1 liter

To prevent the mass from becoming opaque by the precipitation of the carmine, it must be neutralized by the addition of acetic acid until the color is changed from a dark purple to a blood-red. After congealing, the gelatin mixture is cut up and washed in a sieve for several hours to dissolve out the ammonia and acetic acid. It is then dried on paper which has been previously covered with paraffin. For use melt in the water-bath and inject in a warm condition.

Thiersch's Prussian blue gelatin mass.— Make saturated solutions of (1) sulphate of iron, (2) oxalic acid, (3) red prussiate of

potash, (4) and prepare a warm solution of 1 part of gelatin in 2 parts of water. (5) Mix in a separate vessel 12 cc. of solution (1) with 30 grammes of solution (4) at a temperature of 25° C. (6) In another dish mix 60 grammes of solution (4) with 24 cc. of (3), to which add 24 cc. of solution (2), and stir thoroughly. To this last, (6), slowly add solution (5) and stir, with the temperature at 25° C., until the Prussian blue is precipitated. Then heat over water-bath at a temperature of 70° C. and filter through flannel.

Beale's Prussian blue glycerin mass is probably the best of the glycerin masses for students' work, and the formula is as follows:—

Price's glycerin	60 cc.
Ferrocyanide of potassium	2 decigrams
Tincture sesquichlorid of iron	10 drops
Concentrated hydrochloric acid	3 drops
Water	30 cc.

Dissolve the ferrocyanide of potassium in 30 cc. of glycerin, and the sesquichloride of iron in 30 cc. of glycerin, and add drop by drop to the ferrocyanide of potassium solution. Add the water and then the hydrochloric acid.

Starch injection.—Ad Pausch,¹ in 1877, first introduced the method of using starch as an injection substance, and afterward his method was modified by Browning, Della Rossa, Gage, Meyer, and Wikssemiski. Pausch first recommended wheat flour, but later experiments proved that pure starch was superior to flour. Starch is insoluble in alcohol and cold water, and when it is injected in the animal it becomes hard simply by exudation of the liquid containing it. It may be forced up to the capillaries, and, "hardening rapidly, it leaves the vessels flexible." The softness of the starch and its freedom from all gritty material permits the cutting of sections without injury to the microtome knife.

¹ *Arch. f. Anat. Med. Entwick.*, p. 480, 1877. *Amer. Nat.*, XVIII, p. 958, 1884. *Annals Anat. and Surg.*, p. 24, 1884. *Jour. Roy. Mic. Soc.*, p. 979, 1884; pp. 105-106, 1888. *Amer. Mon. Mic. Jour.*, IX, p. 195, 1888.

MASS FOR ORDINARY INJECTION WITH STARCH:—

Dry starch	100 vols.
Aqueous solution of chloral hydrate 2½ per cent . . .	100 vols.
Alcohol 95 per cent	¼ vol.
Coloring material	¼ vol.

Filter through several thicknesses of cambric and stir on the filter to prevent settling.

The addition of the alcohol and the chloral prevents fermentation, and when the mass is injected into the animal the alcohol gives greater fluidity to the mass and rapidly increases the hardening properties after the starch reaches the vessels in the system under study.

Among the colors of most importance for this injection mass are red lead, vermilion, chrome-orange, and ultramarine.

PREPARATION OF THE COLOR:—

Dry color	100 vols.
Glycerin	100 vols.
Alcohol 95 per cent	100 vols.

Grind the color with the liquid in a mortar to prevent the formation of lumps in the mass. Keep in a bottle, and shake before using.

Special injection masses.—For brains and other rapidly perishing organs:—

Starch	100 vols.
Chloral hydrate 5 per cent aqueous solution . . .	50 vols.
Color mixture	75 vols.
Alcohol 95 per cent	25 vols.

To inject fine vessels, first use a diluted form of the stock solution with equal volumes of water, or of the chloral hydrate, and then follow with the stronger solution. Perform the injection quickly, because the exudation takes place so soon that the mass hardens rapidly. In the case of very large vessels, like the aorta, increase the amount of the starch.

Other injection masses.—Some authorities have recommended the use of plaster of Paris, wax, asphaltum, shellac, india ink,

and gum arabic and other substances, but Lee and some other authorities have claimed that "all formulæ which only give opaque masses, or are only suitable for coarse injections for naked-eye study, have been suppressed."

Double injection with gelatin and plaster of Paris may be accomplished by forcing in the animal, first, colored solution of gelatin, and followed by a differently colored plaster of Paris. The latter goes only as far as the capillaries, and we thus have the arteries and larger vessels stained with the plaster of Paris and the capillaries stained with the gelatin.

The Apparatus required for Injection Work

1. Several sizes of cannulas and stopcocks, fitted to a syringe. Or a constant-pressure apparatus.
2. Needles with curved points.
3. No. 10 Chinese silk.
4. Strong twine.
5. Sharp knife or scalpel.
6. Dissecting pans, or similar vessels.
7. Kettle for hot water.
8. Vessel for ice-water.
9. Box, with a close-fitting top, hinged, in which to place the animal for killing with chloroform or ether.
10. Bottle of chloroform or ether.
11. Forceps.
12. Injection masses in large bottles.

There are three methods in practice for injecting fluids into animals:—

1. By means of the syringe with pressure from the hands of the operator.
2. The mechanical or constant-pressure injection.
3. The natural injection, or by introducing pigments into the circulating system of the animal.

By the first system the syringe should be warmed by filling several times with warm water before using the gelatin mass. The syringe should be carefully filled, so that no air will enter

the tissue to be injected, because air will spoil the results. Some practice is required to know just how much pressure to apply to the piston of the syringe. The connections must all fit air-tight, which may be determined by pressing the piston to its lowest position and then, with the fingers over the end of the nozzle,

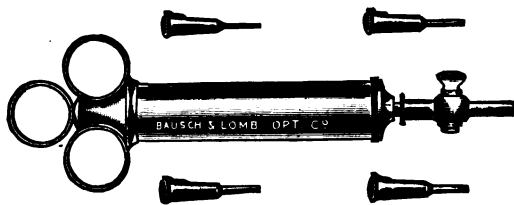


FIG. 116. — Injection Syringe.

drawing the piston out and letting it fly back. If air-tight, it will return quickly to its former position. It is best also to have the rod of the piston graduated, so that the eye may determine whether the injection mass is flowing into the system.

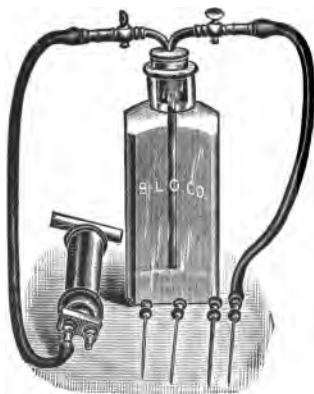


FIG. 117. — Injection Syringe.

Two or three different sizes of these syringes (Figs. 116 and 117) will be found convenient to use with small and large animals. The syringes should hold about 30 cc. and 60 cc. The cannulæ should be 1.6 mm., 0.8 mm., 0.4 mm. in diameter, and there should be on each projections, or arms, on which to tie them to the vessels to be injected (Fig. 118).

The Stroschein injection syringe is made of glass, and it is valuable for bacteriological work because it is easily cleaned and sterilized. The instrument consists of three pieces, — a tube *A* sliding over a smaller tube *B* by means of a rubber connection, and a cannula *C*. The apparatus is placed in operation by slip-

ping *A* as far down on *B* as possible and then inserting the end of the cannula in the injecting fluid. *A* is now returned to its farthest position on *B*, and the suction will cause the

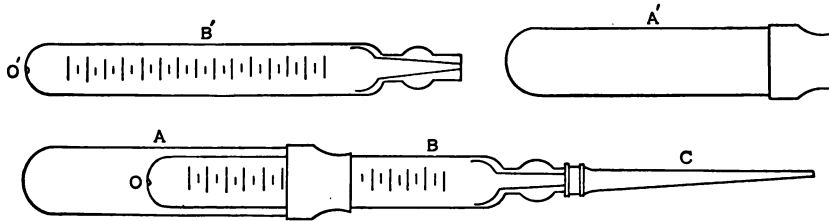


FIG. 118. — Stroschein's Injection Syringe.

liquid to rise in *B*, the air escaping from *B* into *A* by means of the orifice *O*. By a reverse action of *A* the liquid will be expelled from the cannula into the tissues.

The second method is applied by means of the following apparatus, which gives a constant and steady flow of the mass into the system, and, when put into operation, it will work automatically.

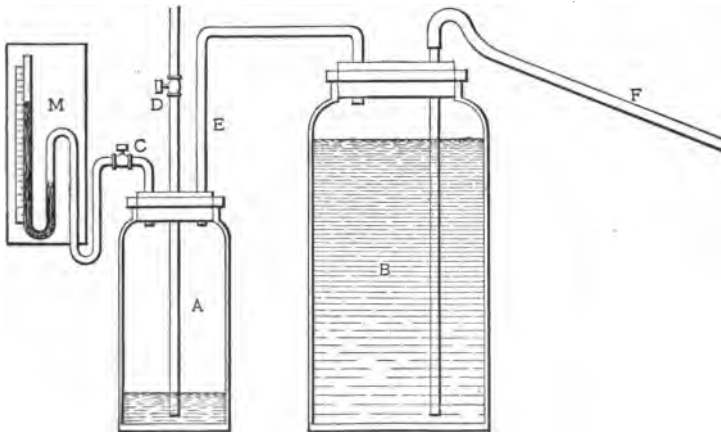


FIG. 119. — Sterling's Constant Pressure Apparatus.

Sterling's constant pressure apparatus. — This consists of a large, wide-mouth bottle *A* (Fig. 119), which has, entering a

closely fitting cork, three tubes, *C*, *D*, *E*. The tube *D* extends to the bottom of the bottle, and the other end connects with the water by means of a faucet. *C* connects with a mercurial manometer *M*, and *E* extends to another bottle *B*, containing the injection mass. From the bottle *B* another tube *F* extends from the bottom to the cannula and the tissues to be injected. The operation of this apparatus is as follows: The stopcock on *D* entering the water-main is opened, and the water flowing into the larger bottle causes the air to press against the injection mass in the bottle *B* and force it into the tissues through the cannula at the other end of the tube *F*. The force of the pressure can be regulated by opening the stopcock in *C* and watching the effects on the mercury in the bent tube on the manometer. If the flow is too great, the stopcock connecting with the water-main must be reduced. In this manner a constant and regular flow of the mass into the system can be maintained.

Killing the animal. — Place in the box mentioned above the animal to be treated to the injection mass and drop in a wad of cotton saturated with chloroform, close down the top, and in about fifteen minutes the animal will be dead. A rabbit is probably the best animal on which the student may experiment with the chance of ultimate success. As soon as dead make an incision through the thorax by cutting the sternal ribs of both sides "sufficiently far from the middle line not to injure the mammary arteries, cutting across the posterior end of the sternum and turning it forward. Slit open the pericardium and make a large incision, by a single cut of the scissors, in each ventricle. All this should be done rapidly, as it is desirable to get rid of as much blood as possible. Pass a ligature round the aorta close to its exit from the heart, and give it a single loose tie. When the bleeding has ceased, sponge the blood from the heart, and pick out any clots which may have formed in the left ventricle, pass a cannula through the incision in the left ventricle into the aorta, tighten the ligature, and knot it firmly. By this operation the whole systemic arteries are injected. The pulmonary arteries may be filled by proceeding

similarly on the right side. The portal vein is readily injected from its branch to the caudate lobe, the cannula being directed toward the main trunk. The injection of the systemic veins is more difficult. The precavals may be filled from the external jugular, the post-caval from the external iliac, the cannula in both cases being directed toward the heart." (*Jour. Mic. Nat. Sci.*)

The work of injection is completed as soon as the extremities of the animal begin to show a decided coloration of the injected mass. Then tie all open vessels and place the animal in cold water. In an hour or so the tissues may be dissected and transferred to alcohol to fix and dehydrate.

Maceration.—The object of this treatment is to separate the tissues from each other by chemicals instead of dissecting with sharp-pointed needles, the chemicals acting on the substances which cement the tissues together and dissolving them so that the tissues easily fall apart. The following are some of the substances used for this purpose :—

Alcohol, 90 per cent in 2 parts of water.

Sodium chloride.

Caustic soda.

Nitric acid.

Osmic acid.

Acetic acid.

Permanganate of potash.

Formaldehyde.

Landois's solution, viz. :—

Saturated solution of neutral chromate of ammonia	5 parts
Saturated solution of phosphate of potash	5 parts
Saturated solution of sulphate of soda	5 parts
Water	100 parts

This formula is suitable for general maceration. (*Arch. f. Mikr. Anat.*, 1885.)

Born's method of reconstructing objects from microscopic sections.—Dr. G. Born¹ describes in detail a very ingenious method

¹ *Arch. f. Mikr. Anat.*, XXII, p. 584, 1883; *Jour. Roy. Mic. Soc.*, p. 634, 1884; *Amer. Nat.*, XVIII, p. 446, 1884.

of constructing models of objects from serial sections. By aid of the camera lucida the outlines of the sections are transferred to wax plates, which are then cut out so as to correspond in outlines as well as dimensions to the sections equally magnified in all three directions. With plates thus prepared, it is only necessary to put them together in the proper order to obtain a complete model. The method is simple and extremely useful, especially in investigating objects with complex internal cavities. Born has made use of the method in studying different parts of the vertebrate head; Swirski, in elucidating the development of the shoulder girdle of the pike; Stöhr, in tracing the development of the skull of the Amphibia and Teleostei; and Uskow, in studying the development of the body cavity, the diaphragm, etc.

Born makes use of three rectangular tin boxes of equal sizes, each measuring $270 \times 230 \times 2\frac{1}{2}$ mm. The sections should be made about $\frac{1}{25}$ mm. thick (never thinner than $\frac{1}{80}$ mm.). If we desire to construct a model of an object from serial sections $\frac{1}{80}$ mm. thick which shall be magnified 60 diameters, then the wax plates must be made sixty times as thick as the sections, *i.e.* 2 mm. thick.

The surface of a plate that could be made in a box of the above dimensions contains 62,100 sq. mm.; and the volume of such a plate 2 mm. thick would therefore be 124.2 cc. The specific gravity of common raw beeswax amounts to 0.96 to 0.97. For use, it requires only to be melted and a little turpentine added to make it more flexible. Thus prepared, its specific gravity is about 0.95; and this number has been sufficiently accurate in all cases. The weight of the wax required to make one plate of the above size will accordingly be 117.99 grammes. The wax having been weighed and melted, the tin box is first filled $1\frac{1}{2}$ cm. deep with boiling water, and then the melted wax poured upon the water. If the water and wax are quite hot, the wax will generally spread evenly over the surface; if gaps remain, they can be filled out by the aid of a glass slide drawn over the wax. As soon as the plate has stiffened, and while it

is still soft, it is well to cut it free from the walls of the tin box, as further cooling of the water and the box might cause it to split. By the time the water becomes tepid, the plate can be removed from the water to some flat support, and left until completely stiffened.

The outlines of the sections are transferred to the plate in the following manner: A piece of blue paper is placed on the plate with the blue side turned toward the wax, and above this is placed a sheet of ordinary drawing-paper. The outlines are drawn on the latter by the aid of the camera lucida, and at the same time blue outlines are traced on the wax plate. The plate can then be laid on soft wood and cut out by the assistance of a small knife. Thus a drawing and a model of each section are prepared. The plates thus prepared can be put together in the proper order, and fastened by the aid of a hot spatula applied to the edges.

Professor H. Strasser¹ gives an improvement and a simplification of the Born plate model system, consisting in the adoption of transparent plates, which are also thinner than any hitherto used.

The apparatus required in the preparation of the plates are an iron roller, 4 cm. in diameter and 30 cm. in length; a water-bath for keeping the wax at a temperature of 60° C.; some strips of tin and brass from 0.2 to 5.0 mm. thick, and a large smooth lithographic stone.

In preparing the wax plates, a piece of the still warm wax is kneaded out in the hands as flat as possible, and having been placed between two leaves of parchment paper kept moistened by turpentine, is rolled out by means of the roller previously warmed. The thickness of the lamella is regulated by the choice of the metal strips placed along the sides of the paper.

When a perfectly flat layer has been thus rolled out, the parchment paper is stripped off and the paper dried between filter-papers. To the surface of these, wax plates are made to adhere by means of gum, for which purpose flour is first rubbed

¹ *Zeitschr. f. wiss. Mikr.*, Vol. III, p. 179, 1886; *Jour. Roy. Mic. Soc.*, p. 171, 1887.

into the plate, or by melting it in by means of a hot roller. The plates thus produced are of fair size, and from $\frac{1}{8}$ to $\frac{1}{4}$ mm. thick. In preparing wax-paper plates to which the section sketch is attached, a very similar procedure is carried out. One of the leaves of the tracing-paper is placed on the lithographic stone damped with turpentine. On the other side is laid a strip of metal, then the wax is spread over the surface, and the second leaf of tracing-paper having been adjusted, a flat lamina is produced by rolling, as before, with the heated roller. The thickness of these plates, paper and all, may not exceed 0.2 mm. Although firm in consistency, they are perfectly flexible, and are cut with sharp knives or scissors quite easily.

*Methods for the Preservation of Marine Organisms*¹

Professor Playfair McMurrich, in writing about the work at the Naples Zoölogical Station, gives the following account of the experiments concerning the preservation of delicate marine organisms made by the conservator of the station, Salvator Lo Bianco: "The last number of the Naples *Mittheilungen*² contains a full description of the methods found most successful for the preservation of the various forms which occur at Naples, and which are undoubtedly applicable to the similar forms found upon our own coasts. An abstract is given in the following pages. It must be fully understood, however, that much depends upon the skill of the preparator, and that want of care and patience will frequently counteract all the advantages to be derived from a good method. All who have had the opportunity of examining specimens prepared by Lo Bianco can appreciate readily the great advantages which may result from a careful application of his methods, and can perceive how greatly we are indebted to him and to Professor Dohrn for their publication.

"Alcohol is, of course, indispensable as a preservative fluid, but certain precautions are necessary in its use. Except in a

¹ *Amer. Nat.*, XXIV, p. 856, 1890.

² *Mittheil. Zoöl. Stat., Neapel*, IX, p. 435, 1890.

few cases it is unnecessary to use it in its full strength, 70 per cent being quite sufficient for preservation, and producing much less contraction and fragility in delicate organisms. Strong alcohol should be reduced with distilled water to the desired strength, ordinary spring-water frequently containing a sufficient amount of carbonate of lime and other substances in solution to give a cloudy precipitate, after a time, which may effectually destroy the appearance of the specimen.

"Furthermore, delicate organisms should first be placed in weak alcohol (35 to 50 per cent) for from two to six hours, the changing of the fluids being effected by a siphon, a small quantity of the weak alcohol being withdrawn and stronger added, until finally the desired strength is obtained. With delicate gelatinous structures the increase in the strength of the alcohol should be as gradual as possible. In many cases it is necessary to use a hardening or fixing agent before the final consignment to alcohol, which is principally useful as a preservative. The most useful fixing agents, according to Lo Bianco, are as follows :—

"**Chromic acid.**—One per cent in fresh water. Objects should not remain in the fluid longer than is necessary to fix them, as they are apt to become brittle. Subsequently they should be well washed with distilled water to prevent the formation of precipitates when placed in alcohol, and also to prevent their taking on too green a tinge from the reduction of the acid.

"**Acetic acid**, concentrated, kills rapidly contractile animals, but must be used with caution, as it produces a softening of the tissues if they are subjected for too long a time to its action.

"**Osmic acid.**—One per cent solution hardens gelatinous forms well and preserves their transparency, but its prolonged action renders the objects brittle and gives a dark-brown tint. Objects hardened in it should be well washed in distilled water before being placed in alcohol.

"**Lactic acid.**—One part to 1000 parts of sea-water fixes larvæ and gelatinous forms well.

"**Corrosive sublimate.**—Saturated solution in fresh or sea water; may be used either hot or cold. It acts quickly, and

preserves admirably for histological purposes. It is especially good combined with copper sulphate, acetic acid, or chromic acid. Objects hardened in it should be subsequently washed in distilled water and in iodized alcohol (the recipe for which is given below), to remove all traces of the sublimate, which in alcohol crystallizes out in the tissues of the organisms and so injures the preparation.

“**Bichromate of potassium.** — Five per cent solution in distilled water hardens gelatinous organisms slowly, without rendering them fragile. It gives, however, a precipitate in alcohol, and discolors the specimen. The discoloration, however, may be removed by adding to the alcohol a few drops of concentrated sulphuric acid.

“**Copper sulphate.** — Five per cent, or 10 per cent, solution in distilled water, used either alone or in combination with corrosive sublimate, kills larvæ and delicate animals without distortion. The objects should be subsequently repeatedly washed with water to remove all traces of the salt, otherwise crystals will form when the object is placed in alcohol.

“Various combinations of these reagents are especially useful, and some of those most serviceable are given below: —

Alcohol and chromic acid.

70 per cent alcohol	}		equal parts
1 per cent chromic acid			

Alcohol and hydrochloric acid.

50 per cent alcohol		100 cc.
Hydrochloric acid, concentrated		5 cc.

Iodized alcohol.

35 per cent or 70 per cent alcohol		100 cc.
Tincture of iodine		2.5 cc.

Chrom-acetic acid, No. 1.

1 per cent chromic acid		100 cc.
Concentrated acetic acid		5 cc.

Chrom-acetic acid, No. 2.

Concentrated acetic acid		100 cc.
1 per cent chromic acid		10 cc.

Chrom-osmic acid.

1 per cent chromic acid		100 cc.
1 per cent osmic acid		2 cc.

Chrom-picric acid.

1 per cent chromic acid	}	equal parts
Kleinenberg's picro-sulphuric acid		

Copper sulphate and corrosive sublimate.

10 per cent solution copper sulphate		100 cc.
Saturated solution corrosive sublimate		10 cc.

Potassium bichromate and osmic acid.

5 per cent solution potassium bichromate		100 cc.
1 per cent osmic acid		2 cc.

Corrosive sublimate and acetic acid.

Saturated solution of corrosive sublimate		100 cc.
Concentrated acetic acid		50 cc.

Corrosive sublimate and chromic acid.

Saturated solution of corrosive sublimate		100 cc.
1 per cent chromic acid		50 cc.

"Frequently great difficulty is experienced in killing an animal without producing a considerable amount of contraction, and in the case of elongated forms, such as Nemerteans, and other worms, without causing them to coil up or become twisted. To avoid this it is expedient to narcotize the animals before killing them, and for this purpose Lo Bianco recommends immersion in weak alcohol. He uses generally a mixture of sea-water 100 cc. and absolute alcohol 5 cc. In other cases 70 per cent alcohol may be carefully poured upon water in which the specimen lies, so that it forms a layer at the surface. It will gradually mix with the subjacent water, and in the course of a few hours will narcotize the animal, so that it may be treated with fixing reagents without fear of contraction.

"Chloral hydrate, 1 to 2 parts sea-water, is also efficient as a narcotizing agent, and has the advantage of allowing a recovery of the animal, if there should be necessity for it, by placing it in fresh sea-water. For some sea-anemones tobacco smoke is useful, the smoke being conducted by a V-shaped tube into a bell-

jar covering the vessel of sea-water in which is the anemone. Certain of these reagents will prove most satisfactory with some animals, others with others. Lo Bianco details the best method for treating the various forms in a second portion of his paper, and an account of some of his methods of procedure, so far as they concern forms which resemble those found upon our coast, may now be presented.

"Sponges. — Direct immersion in 70 per cent alcohol, with subsequent renewal of the fluid, is recommended for the majority of forms. To avoid contraction in the case of the *Halisarcidæ*, they should be left for half an hour in 1 per cent chromic acid, or in concentrated solution of corrosive sublimate for fifteen minutes. To prepare dried specimens the sponges should be washed in fresh water for a few hours, and then allowed to remain in ordinary alcohol for a day, after which they may be dried in the sun.

"Anthozoa. — The first care must be to place the forms belonging to this group in fresh salt water, to allow them to expand, a result which may not be obtained until the following day in some cases. Alcyonarians should be killed with chrom-acetic solution No. 2, withdrawing the water in which they lie, until there is left just enough to cover them, and then adding a volume of the chrom-acetic solution double that of the sea-water. The animals should be removed from this mixture the moment they are killed, since the acid will quickly attack the calcareous spicules, which are important for the identification of the Alcyonaria, and placed in 35 per cent or 50 per cent alcohol, it being well to inject the alcohol into the mouth of the polyps to keep them freely expanded. The preparation should finally be preserved in 70 per cent alcohol.

"Regarding the Actinians no definite rule for preservation can be given. Much of the success of the preparation depends on the form employed, some species contracting much less readily and less perfectly than others. Some may be killed in a fair condition by pouring over them boiling corrosive sublimate, and then, before consigning them to alcohol, treating for a few

minutes with one-half per cent chromic acid. This method may be employed with small forms such as *Aiptasia*. Narcotization may be tried with others. For this purpose, remove from the vessel in which the animals are contained two-thirds of sea-water, and replace it with a 2 per cent solution of chloral hydrate. After a few minutes the fluid is again removed, and cold concentrated corrosive sublimate solution is poured in. Tobacco smoke in some cases, as with *Adamsia*, will act satisfactorily, if followed with vapor of chloroform for two or three hours, after which the animals may be killed in chrom-acetic solution No. 2, and hardened in one-half per cent chromic acid.

"*Edwardsia* may be narcotized by gradually adding 70 per cent alcohol to sea-water in which they are, and subsequently may be killed with hot corrosive sublimate.

"*Cerianthus* should be killed with concentrated acetic acid, placing it as soon as possible in weak alcohol, in which it should be suspended, so that the tentacles may float freely — if necessary, disentangle them.

"Corals should be allowed to expand fully, and then should be killed with boiling solution of corrosive sublimate and acetic acid used in volume equal to that of sea-water containing the coral. The colony should then be transferred to 35 per cent alcohol, some of this fluid being injected into the mouth of each polyp. The injection should be repeated at every change of the alcohol, and the specimens should be preserved in 70 per cent alcohol after washing them well in iodized alcohol.

"**Hydromedusæ.** — For the hydroid colonies the best fixing reagent is hot corrosive sublimate. The smaller Tubularian medusæ should be killed either in the mixture of corrosive sublimate and acetic acid or in Kleinenberg's picro-sulphuric acid. Larger forms may be fixed with concentrated acetic acid, and then allowed to fall into a tube containing the alcohol and chromic acid mixture, in which they are gently agitated and allowed to remain for fifteen minutes, after which they should be transferred to 35 per cent alcohol, and gradually carried to 70 per cent alcohol.

"Small Campanularian medusæ, *e.g.* Eucopa and Obelia, may be killed in the mixture of copper sulphate and corrosive sublimate. *Æquorea* should be killed with concentrated acetic acid, and immediately transferred to chrom-osmic mixture for fifteen to thirty minutes. The same method answers for *Cunina*, while *Liriope* should be treated at once with chrom-osmic from five to twenty minutes.

"Scyphomedusæ are the best fixed with 1 per cent osmic acid, to the action of which they are subjected until they assume a pale brown tint. They should then be thoroughly washed with fresh water before being placed in 35 per cent alcohol, and should be finally preserved in 70 per cent.

"**Siphonophores.** — The forms of this group should be preserved soon after capture, and specimens in good condition should be selected. *Agalma* and similar forms should be killed in a mixture of copper sulphate and sublimate, which should be used in volume equal to or double that of the sea-water in which the animal floats. The mixture should be poured in rapidly, and not over the animal. When killed the specimen should be carefully lifted upon a large horn spatula, and transferred to 35 per cent alcohol for a few hours, and then placed in 70 per cent alcohol. It is recommended to preserve the animals in tubes just large enough to contain the specimens, and placed in a second larger tube. In this way evaporation of the alcohol is prevented, and also injury to the specimen from movements of the liquid is avoided.

"*Physalia* should be placed in a cylinder filled with sea-water, the animal being lifted by the pneumatophore. When well expanded, it is killed by pouring over it the sublimate and acetic acid mixture (one-quarter the volume of the sea-water), and when dead is transferred to a cylinder containing one-half per cent chromic acid, and then, after twenty minutes, to 50 per cent alcohol, and finally to 70 per cent alcohol.

"*Verella* may be killed with chrom-picric or sublimate and chromic acid mixture, and after a few minutes should be transferred to weak alcohol. *Porpita* may be fixed by dropping

Kleinenberg's picro-sulphuric acid into the vessel in which it is contained, and when the blue color begins to change to red it should be transferred to Kleinenberg's fluid, and after fifteen minutes to weak alcohol.

"Diphyes may be killed expanded by hot corrosive sublimate.

"Ctenophora may be killed by throwing them into the chromosmic mixture, where they should remain for fifteen to sixteen minutes, according to the size, and then gradually passing them through alcohol to 70 per cent. A mixture composed of pyroligneous acid, concentrated, 1 vol. ; corrosive sublimate solution, 2 vols. ; one-half per cent chromic acid, 1 vol., is also recommended as a fixative.

"Echinodermata. — Starfish may be prepared with the ambulacral feet in full distension by allowing them to die in 20 to 30 per cent alcohol. Echinoids may be placed in a small quantity of water, and killed with chrom-acetic mixture No. 2, being removed from it as quickly as possible, as acid corrodes the test. To preserve the internal parts it is necessary to make two opposite openings in the test, so that the alcohol may penetrate the interior readily.

"Holothurians — such as *Thyone* and *Cucumaria* — after the tentacles are fully expanded, should be seized a little below the bases of the tentacles by forceps, using a slight pressure, and the anterior portion of the body should then be immersed in concentrated acetic acid. Alcohol (90 per cent) should then be injected into the mouth, and the specimens placed in 70 per cent alcohol. The injection should be repeated each time the alcohol is changed.

"Synapta should be fixed by immersion in a tube containing a mixture of equal parts of sea-water and ether (or chloroform), where they remain completely expanded. They should then be washed for a short time in fresh water, and passed into alcohol, taking care to increase the strength of this very gradually.

"Vermes. — Cestodes, Trematodes, Turbellaria, as well as Nematelminths, are most satisfactorily killed with corrosive

sublimate, either cold or hot. *Sagitta*, however, succeeds best in copper sulphate and sublimate or chrom-osmic mixture.

"*Nemerteans* should be narcotized in a solution of chloral hydrate in sea-water 1 per cent, where they should remain for six to twelve hours. They are then to be hardened in alcohol.

"*Gephyreans* may be narcotized with 1 per cent solution of chloral hydrate in sea-water, or in alcoholized sea-water, three to six hours; or may be killed at once in one-half per cent chromic acid, which last method may be applied to *Hirudinea*.

"*Chaetopods* are best narcotized in sea-water containing 5 per cent of absolute alcohol, or by adding gradually to the surface of the sea-water in which they are contained, a mixture of glycerin 1 part, 70 per cent alcohol 2 parts, and sea-water 2 parts, hardening them subsequently in alcohol. *Chaetopterus* is best killed with 1 per cent chromic acid, in which they should remain for half an hour; while the *Hermellidæ*, *Æphroditidæ*, and the *Eunicinæ* may be killed in cold corrosive sublimate. Some of these, such as *Diopatra*, may, however, be narcotized in alcoholized sea-water.

"*Serpulidæ*, before treatment with corrosive sublimate, should be narcotized in 1 per cent chloral hydrate, which causes them to protrude wholly or partly from their tubes.

"**Crustacea.** — *Cladocera*, *Copepods*, and *Schizopods* may be killed in corrosive sublimate dissolved in sea-water. *Ostrapods* may be thrown at once into 70 per cent alcohol. *Cirripedes* die expanded in 35 per cent alcohol; and if some specimens contract, it is easy to draw out the cirri with forceps. *Amphipods* and *Isopods* may pass directly into 70 per cent alcohol, except the *Bopyrids* and *Etoniscids*, which should be killed in a mixture of equal parts of 90 per cent alcohol and sublimate solution.

"To avoid casting-off of the appendages of the *Decapods*, they should be allowed to die in fresh water, care being taken not to allow them to remain in it longer than is necessary, as it causes a distortion of the membranous appendages.

"*Pycnogonids* will die in one-half per cent chromic acid with the appendages fully extended.

"Mollusca. — Lamellibranchs, Prosobranchs, and Heteropods should be narcotized in alcoholized sea-water. To avoid the closure of the valves of Lamellibranchs on immersion in 70 per cent alcohol, little plugs of wood should be placed between the margins of the valves. The same result may be effected in the case of Prosobranchs by tying the internal edge of the operculum to the shell.

"Of the Opisthobranchs the æolidæ may be best preserved by pouring over them concentrated acetic acid in volumes equal to or double that of the sea-water containing them. Dorids should first be narcotized by gradually adding 70 per cent alcohol to their sea-water, and then killed with concentrated acetic acid or boiling sublimate. The larger forms may be killed in 1 to 5 per cent chromic acid.

"*Pteropods* are preserved well in Perenyi's fluid for fifteen minutes, whence they are passed to 50 per cent alcohol. Gymnosomatous forms should be first narcotized in 1 per cent chloral hydrate, and then killed in acetic acid or sublimate.

"*Decapod Cephalopods* may be fixed directly in 70 per cent alcohol, making an opening on the ventral surface to allow the alcohol to reach the internal parts.

"Bryozoa. — The genera *Pedicellina* and *Loxosoma* may be left for one hour in a 1 per cent chloral hydrate, and then killed with cold corrosive sublimate, washing them immediately afterward. Some species of *Bugula* give good results when the expanded animals are suddenly killed by pouring over them hot corrosive sublimate. With other forms it is possible to preserve them well expanded by adding 70 per cent alcohol gradually to the surface of the water in which they are, or by narcotizing first in weak chloral hydrate or in alcoholized sea-water. The results, however, are uncertain, and depend largely on the skill of the preparator. Brachiopoda may be treated in the same manner as Lamellibranchs.

"Tunicates. — *Clavellina*, *Perophora*, and *Molgula* may be killed with the orifices expanded by immersing them in 1 per cent chloral hydrate for 6 to 12 hours. They should then be killed

in chromic acetic mixture No. 2, and quickly transferred to 1 per cent chromic acid, injecting some of the fluid into the body. After half an hour they should be transferred to 35 per cent alcohol, the injection repeated, and finally to 70 per cent alcohol. Other simple forms may be treated in the same manner, or may require the 2 per cent solution of chloral hydrate, or may be killed by pouring a little 1 per cent chromic acid on the surface of the water in which they are, subsequently hardening in 1 per cent chromic acid. The method recommended for *Perophora* may be employed for compound Ascidians, using, however, corrosive sublimate instead of the chrom-acetic acid mixture.

"*Salpæ* vary considerably in consistency, according to the species, and different methods are consequently required. The denser forms, such as *S. zonaria*, should be placed in a mixture of 100 cc. fresh water and 10 cc. concentrated acetic acid, where they should remain for fifteen minutes. They should then be washed in fresh water for ten minutes, and pass gradually into fresh alcohol; while the soft forms, such as *S. pinnata* and *maxima*, should be placed in chrom-osmic mixture for fifteen to sixty minutes, then washed in fresh water and transferred to weak alcohol.

"*Fishes*. — *Amphioxus* will die with the buccal cirri distended in sea-water alcoholized to 10 per cent. They should then be transferred to 50 per cent alcohol, and gradually to 70 per cent.

"Other forms may be preserved in alcohol (70 per cent), taking care to make a ventral incision, and also to inject the alcohol and to renew it frequently at first. If it is wished to preserve some of the larger Selachians for some months, in order to prepare at leisure the skeleton, the intestines should be removed, and the animals placed in 10 per cent solution of salt.

"Elasmobranch embryos may be fixed in corrosive sublimate, leaving them in the solution for five to fifteen minutes, afterward washing well in iodized alcohol. Embryos of *Torpedo* with the yolk were preserved by immersing in a mixture of

equal parts of 1 per cent of chromic acid and corrosive sublimate for fifteen minutes, and then transferring to alcohol. Transparent fish eggs may be preserved for the purpose of demonstration by subjecting them for a few minutes to the action of the alcohol and hydrochloric acid mixture, and then transferring them to pure alcohol."

There is another method which is sometimes used in this country for the microscopic study of marine forms, which is based upon the gradual displacement of the sea-water in which the animal is living with formaldehyde, and the latter with gelatin, in which the animal is finally mounted. This work may be accomplished by using a glass slip, on which the animal is placed in a drop of sea-water. Over the water is adjusted a cover-glass resting on wax feet, and the formaldehyde is drawn under while the sea-water is gradually extracted from the other side of the cover. The formaldehyde is extracted with gelatin in same manner, and then the cover-glass is sealed permanently.

CHAPTER XVI

POLARIZATION OF LIGHT AND ITS APPLICATION TO BIOLOGICAL INVESTIGATIONS

What is polarized light? To answer this question fully will require a lengthy discussion of physical laws and phenomena bearing on the subject, and this will not be desirable for a book of this character; but it will be possible to enlighten the student by giving some of the general principles on the topic of polarized light.

The law governing the transmission of light states that the ether, or the substance which permeates all space, is vibrated under the influence of the light-emitting body, and that these light waves, or vibrations, take place at right angles to the direction toward which the light is travelling. These vibrations, moreover, are all along the radii of the beam of light.

To polarize the beam of light, therefore, it is necessary to reduce the vibrations to two planes by overcoming the vibrations in all other directions. The beam that comes directly from the sun, and which is not polarized, can be bent in any direction by the interposition of a reflecting surface; but if this beam is first passed through a medium which polarizes the light, the reflecting surface can then bend the rays only in two directions, because the vibrations have been overcome in all other diameters of the original beam except in two. These two polarized vibratory conditions of the ether are termed the "ordinary" and the "extraordinary" rays.

If a piece of Iceland spar, or carbonate of lime, is placed on a sheet of paper containing writing, two images of each letter are seen, which shows that the spar has divided the beam of light coming from the writing into two planes of vibrations,

each having the power of transmitting its image of the letter in different planes. An analysis of this action of the crystal will show that these rays are polarized at right angles to each other.

In order to use this property of the calcium carbonate in connection with the microscope, we must dispense with one of these rays, and this is accomplished by cutting the crystal in half (Fig. 120), along the line BD , and then cementing the halves together again by means of Canada balsam. When the beam of light is passed through the crystal thus prepared, the ordinary ray

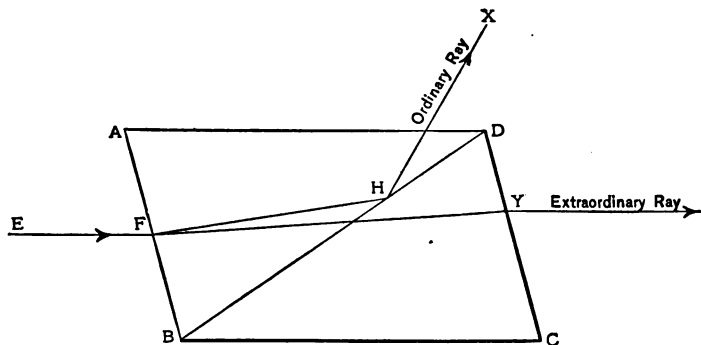


FIG. 120. — Iceland Spar, showing Polarized Light.

strikes the Canada balsam at such an oblique angle¹ that it is totally reflected out of the crystal in the direction HX ; while the extraordinary ray striking the balsam at a less oblique angle, most of it is transmitted through the crystal, and it emerges at the opposite side to which it entered, Y . This preparation of the calcium carbonate is termed Nicol's prisms. The polariscope of the microscope consists of two of these prisms: one is called the polarizer, which is placed beneath the object on the microscope, and the other is called an analyzer, which is placed above the objective or the ocular. They are properly mounted in tubes, as is shown in the illustration

¹ The critical angle of Canada balsam is 69.5° , and when a ray of light strikes it at a greater angle the light is totally reflected.

(Fig. 121), so that they will fit the microscope in the position desired. When the light passes through the first prism, it is reduced, as above stated, into two planes, one called the *ordinary ray* and the other the *extraordinary ray*; and when they strike the film of balsam separating the prisms, the ordinary ray is totally reflected, while the extraordinary ray passes through the second prism and produces the effect called *polarization*. All light must be excluded from the stage of the microscope except that which comes through the polarizer, because, if this precaution is not taken, the field will not be sufficiently darkened for satisfactory results. When the polarizer and analyzer are

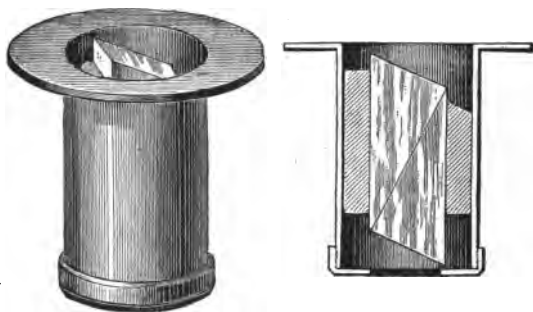


FIG. 121. — Zeiss' Polarizer.

in their places on the microscope, it will be found that by turning the analyzer, there is a position when the largest quantity of the light reaches the eye, and also a position when the light is entirely cut off. The first position is when the prisms are parallel to each other, and the latter when they are almost at right angles.

If an object is now placed on the stage of the microscope when the polariscope is in position, the object remains invisible when the analyzer and polarizer are at right angles to each other. But if the object has polariscopic properties, it will become partly visible, because it will so affect the beam of light passing through the first prism as to polarize the light again, and the object will be partially visible in the analyzer, since the

polarizer ray is changed to another plane by the interposed object. These objects are called *polariscopic* — such as thin films of selenite, certain woody tissues of plants, starch grains, sections of horn; some animal and plant hairs belong to this class. Beautiful colors become visible in the microscope when these polariscopic objects are interposed between the polarizer and analyzer. To have these color effects the object must not be too thick or too thin. If the substance is quite thick the light will assume its white condition, and if too thin a gray result is secured on a black ground, so that an effort must be made to obtain intermediate thicknesses.

Adjustment of the polariscope. — Swing out the reflector and the Abbe illuminator and take out the diaphragm. Insert the polariscope beneath the stage in the position occupied by the diaphragm. Place back the illuminating apparatus. Put the analyzer above the ocular. Now shade the stage so that no ray of light can reach it except that which is reflected through from the Abbe illuminator beneath. The analyzer must be adjusted to the ocular by raising or lowering until the field becomes clear and sharp to the outer edge. Now turn the analyzer until the brightest amount of light comes through from the stage and determine this relative position of the analyzer and polarizer. Then turn the analyzer so that all light is excluded and mark this position. Upon examination it will be found that these two positions are 90° apart. When the greatest amount of light is transmitted to the eye through the analyzer we see the extraordinary ray, and this is the position for observing the image of the object it is desired to examine under the influence of the polarized ray.

The polariscope is used to answer among others the following questions: (1) Is an object simply refractive in its properties, that is optically homogeneous? (2) Has the object the power of double refraction? (3) Are there minute crystals in the section which are not visible under the microscope with the use of the ordinary white light unpolarized? (4) Is it possible to distinguish the crystals in rock sections and determine the composition of the

rock? (5) Are the beautiful and striking effects produced by the following objects characteristic: starch, fibres of plants, hairs of animals and plants, cellular tissue, muscular tissue of animals, sections of bone, hoofs, horn?

Selenite should comprise part of the polariscope outfit because it is useful at times in increasing the thickness of certain sections which are too thin to produce the color effects. The selenite may be introduced at any position between the polarizer and the analyzer. This selenite stage, as it is sometimes called, also greatly aids in the study of substances which have the power of only single refraction, or *mono-refrigent*, such as glass which has been strained, and other substances which have not the correct thickness to cause polarization. In such cases the selenite produces a colored field in which the crystals are brought out clear and sharp.

Mohl has done much valuable work with the polarizers in investigating the effects of the light on vegetable organisms, and Messrs. Griffith and Henfrey thus sum up the results secured by him:¹—

“It is easy to ascertain whether an organic body shows positive or negative colors, by comparing its color, when seen with a plate of gypsum in a certain definite position, with the color given under the same circumstances by a strip of glass brought into a state of tension by a slight bending, or with the colors of a suddenly cooled globule of glass. In this way the author determined that the fibres of a spiral vessel displayed negative colors, and the laminae of a starch corpuscle positive colors, and then applied these organic structures, by comparison, for ascertaining the properties of other objects. The objects to be examined should be mounted in a liquid or other substance, rendering them as transparent as possible, such as glycerin, Canada balsam, or an essential oil.

“When ordinary globular or cylindrical cellular tissues are viewed by cross-sections, their substance is seen to be doubly refractive; for when the prisms cross, the circular sections of

¹ “Micrographic Dictionary,” Griffith and Henfrey, pp. 612, 613.

cell walls appear like rings of bright light on a black ground, but with the ring divided into four quadrants by dark stripes, as if a black cross lay over it; when the prisms are placed parallel, the parts of the section previously bright appear dark, and *vice versa*, on a bright field. If a section of polyhedral cellular tissue is viewed in the same way, the appearances are somewhat different, since the cut edges are here straight lines, variously inclined toward the prisms; those which are perpendicular to the prisms are invisible, while those standing obliquely are bright in their whole length. In general, cell membrane acts more powerfully on the light the denser its substance, and soft collenchymatous tissues are far less powerfully doubly refractive than wood cells. When the cells have walls, much thickened, it is common for the primary cell membrane to be much more powerfully refractive than the secondary layers.

* * * * *

"Let us suppose that between the lower prism and the object is placed a plate of selenite, giving a red field; the plate is then rotated so that its neutral axes are at an angle of 45° with the prisms. A section of a cylindrical vegetable cell will be seen to be divided into four quadrants: the two alternate quadrants, whose middle lines correspond to the neutral axes of selenite, are either blue or green, the other two yellow or red; if the selenite is then rotated so that its neutral axes are perpendicular to the prisms, the colors will be all lost; but on continuing the rotation, they appear in the reverse order—what was blue appearing yellow, and *vice versa*. When the walls are rectilinear, all the cell walls perpendicular to one of the prisms will give the color of the field, all those which run parallel with one of the neutral axes of the selenite plate, or form no great angle with it, will be blue, those parallel with the other axis yellow.

"It is found that the vegetable structures fall into two classes in reference to these colors, in one of which classes all layers lying obliquely in the direction of a right-wound screw are tinged blue and yellow, those oblique in the opposite direction yellow

or red; in the other class, the colors under the same conditions are just the reverse; so that one class are optically positive, the other optically negative.

"The optically negative are the ordinary cell-membranes of the internal organs of plants, whether in their natural condition or cellulose, purified by the help of nitric acid and chlorate of potash. Collenchyma, horny endosperm-cells, the gelatinous cells of Algæ, etc., all agree in this property. Optically positive colors are given by cell membranes of periderm and cuticular layers of epidermal cells. The contrast of the positive and negative colors of the cuticle and other parts of the cell wall is well seen in the epidermis of Aloe. The diversity of coloring under polarized light here corresponds to the diverse behavior under treatment with iodine after maceration in solution of potash (secondary deposits).

"The longitudinal sections of all behave like the cross-sections; but the appearances are not so clear. When side views of the surface of cells are obtained, the phenomena are very varied; but these are not best seen in vessels or ducts when the thickening layers are in the form of spiral bands. Thus, if one of the spiral vessels of *Musa* is placed (its spiral somewhat drawn apart) with its long axis perpendicular to one of the prisms, the fibres on the upper side turn to the left, those on the under side toward the right; and when the selenite plate is interposed, they exhibit the complementary colors. When the side walls of cells have obscure striation, as in the cells of conifers, the liber cells of *Apocynæ*, etc., the membrane gives evidence of its fibrillar structure by the yellow or blue color developed with the selenite plate. If fibres of a spiral vessel cross at right angles, and they are pressed together, they neutralize one another when they cross: when the prisms are used alone, the crossing points are black, the rest of the fibres white; when the selenite plate is interposed, the crossing points exhibit the color of the field, and the uncrossed portions of the fibres are blue or yellow, according to position.

"The vicinity of a round bordered pit, as in the wood cells of

Pinus, exhibits a black cross when seen perpendicularly by polarized light. The black cross and the colors exhibited by starch are well known. Chlorophyl does not seem to act on polarized light, nor the primordial utricle of cells, except a trace when contracted by weak alcohol.

"The polarization apparatus is exceedingly useful for the detection of crystals (Raphides) in vegetable tissues, when they are so small as to be readily overlooked; and the larger kinds form beautiful objects with, and often without, the selenite plate."

By an elaboration of these experiments with sections of various kinds of plants, much useful information will be gleaned, which will enable the microscopist to arrive at conclusions in no other way attainable. The limit and the character of this work will not permit of more extended discussion of this interesting subject, but the director of the laboratory will give the students much valuable, as well as interesting, information, if he will require them to make frequent tests with the polariscope, while studying cross-sections of the plants. For a full account of polarization in microscopical investigation, the student is referred to the writings of Dippel, Nägeli, and Schwendener.

CHAPTER XVII

USEFUL FORMULÆ AND TABLES

THERE are many useful formulæ and tables which are of importance to microscopists, scattered through the pages of journals and books devoted to biological research, and their enumeration would much more than fill the leaves of a book larger than this. The attempt, therefore, will be made to mention only a few of the more important:—

Cleaning glass.—1. Place in a wide-mouth bottle a strong solution of borax and washing-soda, or caustic potash. The glasses placed in this will soon become cleaned of gums and other foreign matters.

2. Dichromate of potash	10 grammes
Hot water	50 cc.
After cooling, add slowly sulphuric acid . . .	50 cc.

First remove the covers from slides by heating two or three seconds over Bunsen's burner; place the glasses in a porcelain dish, pour over them the fluid, and heat on water-bath for ten minutes. When clean, wash the glasses and immerse in solution of dilute caustic soda, warm for five minutes, place in spirits, and dry.

3. "One box or pound of crude potash dissolved in one gallon of water. Bring to a boil. Dip the old gelatin plates in this, in a dipping-rack that will keep them apart. Dip with movement, and when the film is gone, remove the rack and glass into another vessel of warm water, and then wash well in running water. Take them, one at a time, and rub with rag covered with pulverized cuttlefish bone. Put them in a rack to dry and then pack away for use. When the plates are to be used, polish

4. Dr. F. Knauer's method: The slides are placed in a vessel containing about half a liter of 10 per cent solution of lysol, and boiled for twenty to thirty minutes. The still seething vessel is then placed under the faucet and the water allowed to flow in until the solution becomes quite clear, when the slides are taken out and dried.

6. Place in a wide-mouth jar a mixture of benzin, turpentine, and benzol, and let the slides remain in this all night. Take out and polish with a soft cloth. (F. L. JAMES.)

7. Canada balsam: Heat the solid balsam until brittle when cold, and then dissolve in either benzol, xylol, chloroform, or turpentine to the required consistency. The xylol solution is generally considered to be the best, but it sets more slowly than the benzol solution.

9. To prepare glycerin jelly for delicate mounting: Dissolve transparent isinglass in a sufficient quantity of distilled water to make a stiff jelly. When at ordinary temperature of working room add one-tenth as much good glycerin and a little solution of borax, carbolic acid, or camphor water. Filter while hot through washed muslin, and subsequently add a little alcohol to improve its working. (*Amer. Mic. Jour.*, 1881.)

Glycerin	.	.	.	.	.	.	I part
Alcohol (96 per cent)	.	.	.	.	.	.	I part
Water	,	.	.	.	.	.	I part (A. B. AUBERT.)

11. Glycerin jelly:—

Glycerin		120 grammes
Gelatin		30 grammes
Water		60 grammes

Dissolve the gelatin in warm water, add the glycerin, filter.
For vegetable and animal tissue. (A. B. AUBERT.)

12. Farrant's medium for delicate plants or animal tissue:

Gum arabic		30 grammes
Glycerin		30 grammes
Arsenious oxide		0.1 gramme
Water		30 cc.

To be kept in a stoppered bottle with a lump of camphor.

Fixing and Clearing Formula

13. Mayer's albumen fixing method (Lee):—

White of egg		50 cc.
Glycerin		50 cc.
Salicylate of soda		1 gramme

Shake well together, and filter into a clean bottle.

The following is a modification of Mayer's method, given in the *Amer. Mic. Jour.*, p. 119, 1895:—

"A layer of Mayer's albumen was spread on the slide, and the sections arranged. Then a wash $\frac{3}{4}$ per cent collodion was spread over the surface evenly with a camel's-hair brush. This is allowed to dry, which takes place in about one minute, but a longer time does no harm; practically one slide dries while the next is being prepared. During the drying many small air bubbles appear, the presence of which indicates the right degree of dryness; these do not cause any inconvenience, as they disappear during the subsequent processes. When dry the slide is put up *without heating* into a jar of xylol or benzin for half an hour or more to dissolve the paraffin. A stay of several hours will not injure the tissues. The paraffin may be removed in three to five minutes by constantly moving the slide in the benzin. The benzin or xylol is removed by 95 per cent alcohol, and the sections are stained and mounted as desired."

Preserving Medium for Plants

14. Formalin or formol preserving medium:—

Formaldehyd	40 parts
Water	100 parts

Chlorophyl and all delicate parts of plants are well preserved in this solution, and the colors are not destroyed — some flowers are preserved.

Photographic Developing Solutions

15. Carbutt's pyrogallic acid developer:—

No. 1. Pyrogallic Acid Stock Solution

300 cc. distilled or ice water	10 ounces
1 gramme oxalic acid	15 grains
2 grammes bromide potassium	30 grains

Then add Schering's pyrogallic acid 1 ounce (30 grammes), and water to make 16 fluid ounces (480 cc.).

No. 2. Stock Soda Solution

300 cc. water	10 ounces
120 grammes soda sulphite crystals	4 ounces
60 grammes soda carb. crys. (or dry grain 1 ounce)	2 ounces
30 grammes potash carbonate	1 ounce

Dissolve, and add water to make measure 16 fluid ounces (480 cc.).

No. 3. Bromide Solution

14 grammes bromide of sodium or potassium	$\frac{1}{2}$ ounce
150 cc. water	5 ounces

Dilute 2 ounces of stock No. 2 with 7 ounces of water for cold weather, and 10 to 12 of water in summer. To 3 ounces of dilute No. 2 add $1\frac{1}{2}$ to $2\frac{1}{2}$ drachms (6 to 10 cc.) of No. 1. The more pyro, the denser the negative, and *vice versa*. No yellowing or fogging need be apprehended if our directions are followed. Development should be continued until the image seems almost buried, then wash and place in fixing-bath.

For *instantaneous exposures* take for a 5×8 or $6\frac{1}{2} \times 8\frac{1}{2}$ plate 3 ounces of dilute No. 2. Lay the plate to soak in this, and cover pan. Put 2 drachms of No. 1 into the graduate, and 3 drops of bromide solution. Pour the soda solution off of the plate into the pyro and back over the plate; let development proceed, and examine occasionally. Keep solution in gentle motion over the plate.. A *very* short exposure may take ten minutes to fully develop. If the image is not fully brought out this time, add to developer in pan three times its bulk of water, and let plate lie in it covered over for half an hour or more if necessary, until full development is attained, then wash and proceed as directed under head of developer.

Cramer's Pyrogallic Developer

16. Alkaline solution : —

177.6 cc.	water	60 ounces
142	grammes carbonate of soda crystals	5 ounces
284	grammes sulphite of sodium crystals	10 ounces

A smaller quantity of sulphite will produce a warm tone, a larger quantity a gray or bluish black tone.

The alkaline solution must be kept in well-stoppered bottles.

If the negative shows a yellow stain make a fresh solution, or try another lot of sulphite of sodium.

Pyro solution : —

Dissolve 1 drachm sulphite of sodium crystals in 6 ounces of distilled water, add acetic acid until the solution turns blue litmus-paper red, and finally add 1 ounce of pyrogallic acid.

For the developer use the following proportions : —

3.7 cc.	pyrogallic acid solution	1 drachm
29.5 cc.	alkaline solution	1 ounce
59.2 cc.	tepid water (for winter use)	2 ounces, or
88.8 to 148 cc.	cold water (for summer use)	3 to 5 ounces

17. Eastman's pyrogallic developer (for films) : —

No. 1

14.2 grammes	pyrogallic acid	$\frac{1}{2}$ ounce
1.2 cc.	nitrous or sulphurous acid	20 minims
947 cc.	water	32 ounces

No. 2

170 grammes	sulphite of soda crystals	6 ounces
113.6 grammes	carbonate soda crystals	4 ounces
947 cc.	water	32 ounces

To develop take : —

29.6 cc.	No. 1	1 ounce
29.6 cc.	No. 2	1 ounce
59.2 cc.	water	2 ounces

18. Eastman's pyrogallic developer (for plates): —

No. 1

170 grammes	sulphite of soda crystals	6 ounces
28.4 grammes	pyrogallic acid	1 ounce
947 cc.	water	32 ounces

No. 2

113.6 grammes	carbonate of soda crystals	4 ounces
947 cc.	water	32 ounces

To develop take : —

29.6 cc.	No. 1	1 ounce
29.6 cc.	No. 2	1 ounce
78.8 to 118 cc.	water	3 to 4 ounces

In warm weather use more water; in cold, less.

19. Hammer's pyrogallic developer : —

No. 1

Pure water	900 cc.
Sulphite of soda crystals	150 grammes
Carbonate of soda crystals	75 grammes

No. 2

Pure water	720 cc.
Oxalic acid	1 gramme
Pyrogallic acid	30 grammes

To develop take :—

No. 1	30 cc.
No. 2	15 cc.
Pure water	90 to 180 cc.

More water may be used in warm weather, and less in cold weather.

20. Seed's pyrogallic developer :—

A

296 cc. water	10 ounces
14.9 grammes sulphite of soda crystals	$\frac{1}{2}$ ounce

Add enough pure acetic acid to this to turn blue litmus-paper slightly red, then add pyrogallic acid 1 ounce.

B

473.6 cc. water	16 ounces
113.6 grammes sulphite of soda crystals	4 ounces

C

473.6 cc. water	16 ounces
113.6 grammes sal. soda crystals	4 ounces

To develop take :—

14.8 cc. A	$\frac{1}{2}$ ounce
29.6 cc. B	1 ounce
29.6 cc. C	1 ounce
236.6 cc. water	8 ounces

For double-coated plates use 18 ounces of water.

21. Stanley pyrogallic developer :—

No. 1. Alkaline Solution

2368 cc. pure water	80 ounces
186 grammes sulphite of soda crystals	6 oz. (Troy)
186 grammes carbonate of soda crystals	6 ounces

No. 2. Pyrogallic Solution

2368 cc. water	80 ounces
1 gramme sulphite of soda	$\frac{1}{2}$ drachm
28.4 grammes pyrogallic acid	1 ounce

For use, mix No. 1 and No. 2 in equal parts.

Hydrochinon Developers

22. Carbutt's developer:—

A

Warm distilled water	600 cc.
Sulphite of soda crystals	120 grammes
Sulphuric acid	4 cc.
Hydrochinon	23½ grammes
Bromide of potassium	2 grammes
Water to make up to	960 cc.

B

Carbonate of potash	60 grammes
Carbonate of soda crystals	60 grammes
Water to make	960 cc.

C. Accelerator

Caustic soda	30 grammes
Water	300 cc.

For underexposure a few drops of this are added to developer.

D. Restrainer

Bromide of potassium	14 grammes
Water	150 cc.

For developer take	A	B	Water
For instantaneous exposures	30 cc.	30 cc.	120 cc.
For portraits	30 cc.	30 cc.	150 cc.
For landscapes, Sen. 20-27	30 cc.	15 cc.	90 cc.
Full exposures, Sen. 16-20	30 cc.	25 cc.	120 cc.
For lantern slides	30 cc.	25 cc.	120 cc.

23. Seed's developer:—

A

28.4 grammes hydrochinon	1 ounce
142 grammes sulphite of soda crystals	5 ounces
0.6 gramme bromide of potassium	10 grains
1634 cc. water (ice or distilled)	55 ounces

B

11.6 grammes caustic potash		180 grains
296 cc. water		10 ounces

For the developer take :—

118.4 cc. A		4 ounces
14.8 cc. B		$\frac{1}{2}$ ounce

The temperature of room should be from 21° to 24° C.

Eikonogen Developer

24. Cramer's developer :—

No. 1

1776 cc. distilled water		60 ounces
85.8 grammes sulphite of soda crystals		3 ounces
28.6 grammes eikonogen		1 ounce

Boil for a few minutes. After cooling, pour into a bottle and keep well corked.

No. 2

984 cc. water		40 ounces
28.6 grammes carbonate of potash		1 ounce

For the developer, use of solution No. 1, 3 ounces; solution No. 2, 1 ounce. In hot weather dilute with an equal quantity of cold water.

25. Eastman's developer :—

No. 1

85.8 grammes sulphite of soda crystals		3 ounces
28.6 grammes eikonogen		1 ounce
1776 cc. water		60 ounces

No. 2

85.8 grammes carbonate of potash		3 ounces
888 cc. water		30 ounces

To develop take :—

59.2 cc. No. 1		2 ounces
29.6 cc. No. 2		1 ounce
59.2 cc. water		2 ounces

Eikonogen-Hydrochinon Developers

26. Carbutt's eikonogen and hydrochinon developer for orthochromatic plates, "celluloid" films, and transparencies:—

A

Distilled water	600 cc.
Sulphite of soda crystals	120 grammes
Eikonogen	22 grammes
Hydrochinon	10½ grammes
Water to make up to	960 cc.

B

Distilled water	600 cc.
Carbonate of potash	60 grammes
Carbonate of soda crystals	60 grammes
Water to make up to	960 cc.

DEVELOPER

For developer take	A	B	Water
For instantaneous exposures	30 cc.	30 cc.	120 cc.
For landscapes, Sen. 20-27	30 cc.	15 cc.	90 cc.
Full exposures, Sen. 16-20	30 cc.	25 cc.	120 cc.
For lantern slides	30 cc.	25 cc.	120 cc.
Full exposures	and 2 to 6 drops restrainer D to each ounce developer. (See below.)		

NOTE.—More of A will increase density, more of B will increase detail and softness. Temperature of developer should not vary much below 18° C. nor above 24° C. The after treatment is the same as with any other developer.

27. Seed's eikonogen-hydrochinon developer:—

No. 1

42.6 grammes sulphite of soda	1½ ounces
16.8 grammes eikonogen	240 grains
3.9 grammes hydrochinon	60 grains
947 cc. distilled water	32 ounces

No. 2

113.6 grammes carbonate of soda	4 ounces
947 cc. water	32 ounces

To develop take:—

59.2 cc. No. 1	2 ounces
29.6 cc. No. 2	1 ounce
29.6 cc. water	1 ounce

For double-coated plates use 5 ounces of water.

28. Metol developer:—

Cramer's Developer

5.2 grammes metol	80 grains
85.2 grammes sulphite of soda crystals	3 ounces
28.4 grammes carbonate of soda crystals	1 ounce
1 gramme bromide of potassium	12 grains
2368 cc. water	80 ounces

Used for developing underexposed plates.

29. Glycin developer:

Jules Fuerst's Formula

A

Sulphite of soda crystals	125 grammes
Potassium carbonate	50 grammes
Glycin	50 grammes
Hot water	1000 cc.

B

Potassium carbonate	125 grammes
Water	1000 cc.

C

Sulphite of soda crystals	125 grammes
Potassium carbonate	250 grammes
Glycin	50 grammes
Hot water	1000 cc.

C is a concentrated one-solution developer.

For normal exposure, take 1 part of A, 2 parts of B, and 1 part of water, or 1 part of C diluted with 3 times its bulk of

water, or 1 to 6 for underexposure. For overexposure, 3 parts of A, to 2 parts of B and 3 parts of water. For underexposure, developer should be freely diluted to give time for developer to act on slightest light impressions without "plugging" high lights. For unknown exposures, take 1 part of B, 2 parts of A, and 2 parts of water, to which add 15 drops of bromide of potassium, 10 per cent. If the details appear in less than thirty seconds, the plate is overexposed. Any tendency to harshness must be remedied by addition of more potash and further dilution. (*International Annual* for 1902.)

Fixing-baths

30. Carbutt's new acid fixing and clearing bath:—

Sulphuric acid		4 cc.
Hyposulphite of soda		480 grammes
Sulphite of soda		60 grammes
Chrome-alum ¹		30 grammes
Warm water		1920 cc.

Dissolve the hyposulphite of soda in 1440 cc. of water, the sulphite of soda in 180 cc. of water, mix the sulphuric acid with 60 cc. of water, and pour slowly into the sulphite of soda solution, and add to the hyposulphite; then dissolve the chrome-alum in 240 cc. of water and add to the bulk of solution, and the bath is ready. This fixing-bath will not discolor until after long usage, and both clears up the shadows of the negative and hardens the film at the same time.

After negative is cleared of all appearance of silver bromide, wash in running water for not less than half an hour, to free from any trace of hypo solution. Swab the surface with wad of wet cotton, rinse, and place in rack to dry spontaneously.

31. Cramer's fixing-bath:—

No. 1

908.8 grammes hyposulphite of soda		32 ounces
2841.6 cc. water		96 ounces

¹ During cold weather use only half the quantity of chrome alum in above.

No. 2

113.6 grammes sulphite of soda crystals	. . .	4 ounces
14.8 cc. sulphuric acid	. . .	$\frac{1}{2}$ ounce
56.8 grammes powdered chrome-alum	. . .	2 ounces
947 cc. water	. . .	32 ounces

Pour No. 2 into No. 1.

32. Hammer's fixing-bath :—

10 cc. sulphuric acid	. . .	3 drachms
113.6 grammes sulphite of soda	. . .	4 ounces
2960 cc. water	. . .	about 100 ounces

When this is about half dissolved, add 2 pounds of hyposulphite of soda; after the hyposulphite of soda is dissolved, add from 1 to 2 ounces of chrome-alum dissolved in 20 ounces of water; then add enough water to make 160 ounces.

33. Seed's fixing-bath :—

No. 1

907 grammes hyposulphite of soda	. . .	2 pounds
113.6 grammes sulphite of soda crystals	. . .	4 ounces
2841.6 cc. water	. . .	96 ounces

No. 2

56.8 grammes chrome-alum	. . .	2 ounces
7.8 cc. sulphuric acid	. . .	$\frac{1}{4}$ ounce
947 cc. water	. . .	32 ounces

Pour No. 2 into No. 1 while stirring rapidly.

Intensification Solutions for Photographic Plates

34. Carbutt's intensification solution.

With correct exposure and development, intensification need never be resorted to. The following formula is, however, very effective, and the most permanent of all methods :—

No. 1

16 grammes bichlorid of mercury	. . .	240 grains
16 grammes chlorid of ammonia	. . .	240 grains
600 cc. distilled water	. . .	20 ounces

No. 2

16 grammes chlorid of ammonia	240 grains
600 cc. water	20 ounces

Let the plate to be intensified wash for at least half an hour, then lay in a 5 per cent solution of alum for ten minutes, and again wash thoroughly; this is to insure the perfect elimination of the hypo. The least trace of yellowness after intensifying shows that the washing was not sufficient.

Flow sufficient of No. 1 over the negative to cover it, and allow to either partially or entirely whiten; *the longer it is allowed to act the more intense* will be the result. Pour off into the sink; rinse, and flow over No. 2, and allow to act one minute; wash off, and pour over or immerse in dilute ammonia water (1 drachm strong ammonia in 8 ounces of water) until changed entirely to a dark brown or black. Wash thoroughly and dry.

35. Cramer's intensification solution:—

No. 1

Bichlorid of mercury	saturated solution
Hydrochloric acid, to each ounce	1 drop
Water	20 parts

No. 2

28.6 grammes sulphite of soda	1 ounce
592 cc. water	20 ounces

Immerse negative in first, until evenly whitened, then rinse and apply the sulphite solution until the negative is well blackened, wash and dry.

36. Seed's fixing-bath:—

28.6 grammes mercuric chlorid	1 ounce
28.6 grammes potassium bromid	1 ounce
1480 cc. water	50 ounces

Whiten the negative in this solution, wash and immerse in equal parts of a saturated solution of sulphite of soda and water. Wash and dry.

Reduction Solutions

37. Carbutt's reduction solution. (Farmer's formula.)

No. 1

28.6 grammes ferricyanid of potassium	. . .	1 ounce
473 cc. water		16 ounces

No. 2

28.6 grammes hyposulphite of soda	. . .	1 ounce
473 cc. water		16 ounces

Immerse the negative in the hyposulphite solution, to which have been added a few drops to each ounce of the above ferricyanid solution. The speed of reduction depends on the quantity of ferricyanid present. Wash thoroughly.

38. Cramer's reduction solution : —

No. 1

28.6 grammes red prussiate of potassium	. . .	1 ounce
473 cc. water		16 ounces

No. 2

28.6 grammes hyposulphite of soda	. . .	1 ounce
473 cc. water		16 ounces

No. 1 is affected by light and must be wrapped with opaque paper and kept in a dark place when not in use.

Mix 8 ounces of No. 2 and one ounce of No. 1 and use in subdued daylight. Wash thoroughly to eliminate the hyposulphite of soda. A dry negative must be first soaked in water, but a negative just fixed may be transferred at once to the reducer provided all of the hyposulphite fixing-bath has been well washed out. Keep the vessel in motion while reducing. After washing immerse the plate in a solution of

57 2 grammes sulphite of soda crystals	. . .	2 ounces
592 cc. water		20 ounces

to stop the action of the reducer, then wash thoroughly in running water for an hour.

39. Permanganate of potassium reducer :—

Permanganate of potassium	0.5 gramme
Sulphuric acid	1 cc.
Water	1000 cc.

This solution keeps for a long time and may be applied to the negative after fixing, even if the same has not been thoroughly washed, as it destroys all traces of fixing soda by oxidation. During the process the tray must be kept in motion. (HENRY DIETRICH, in Anthony's *Photo. Bul.*, Vol. XXXI.)

40. Persulphate of ammonia reducer :—

1 gramme persulphate of ammonia	15 grains
28.4 cc. water	1 ounce

When the desired effect is produced immerse in the following, after washing, a 10 per cent solution of sulphite of soda; wash and dry. Highly indorsed by some photographers. (H. S. LAUDER, in Wilson's *Photo. Mag.*, Vol. XXXVII.)

Another :—

Persulphate of ammonia	2 to 3 per cent.
Glycerin to slightly thicken.	

Another :—

.07 gramme permanganate potassium	1 grain
113.6 cc. water	4 ounces
2 drops sulphuric acid	2 drops

After reduction clear with 2 drops of oxalic acid in 1 ounce of water.

41. Monochromatic light filters :—

Dr. Nagel in Freiburg gives a very interesting description of how to make monochromatic filters for different physiological purposes, which are undoubtedly of importance for all ortho-chromatic work, the three color processes, and all color sensitive plates. The several color-filter solutions are as follows :—

Orange filter for the spectrum district between C and D.—
Prepare a solution of acetate of copper, add a few drops of

acetic acid, and then, drop by drop, a concentrated saffranin solution, until the solution will admit no more violet, blue, green, and yellow light.

Yellow-ray filter. — Add to a saturated acid solution of acetate of copper a saturated acidified solution, "Orange G." The liquid has a brown appearance, and passes only a small stripe of yellow light.

Green-yellow filter. — To a saturated solution of bichromate of potassium acidified with acetic acid add crystals of acetate of copper, and heat the solution. The green liquid passes only monochrome green light.

Green-ray filter. — To a saturated solution of bichromate of potassium add in drops a solution of ammonia carbonate of copper until the red to yellow rays are absorbed. To this solution add a few drops of alkaline fluorescent solution.

Blue-ray filter. — A weak solution of methyl-green is mixed with acetate of copper solution until no red light passes.

Violet-ray filter. — Ammonium oxide of copper solution concentrated and mixed with 7 parts of water gives, in combination with a solution of permanganate of potash, a spectrum which will admit only the light between F and G.

Red filter. — Dissolve carmine in a solution of carbonate of lithium, and mix the solution with picric acid. (HENRY DIETRICH in Anthony's *Photo. Bul.*, Vol. XXXI.)

In order to make the references to the lines C, D, F, and G mentioned above clear the following table is given, taken from *Roy. Mic. Jour.* of February, 1898 :—

Fraunhofer Lines	Wave Lengths (Å)
A	759
B	687
C	656
D	589
E	527
F	486
G	430
H	397

Distribution of colors (after Listing):—

	λ
Red	723-647
Orange;	647-586
Yellow	586-534
Green	534-491
Bright blue	491-455
Blue violet	455-424
Violet	424-397

42. Printing formulæ for positives. Carbutt's vinco:—

The light and exposure.— Any artificial light can be used in an ordinary room, turn down flame of gas or oil light, open package of vinco, place piece of paper over negative in printing-frame (the sensitive side may be known by its tendency to curl inwards), turn up light, and expose for an average good negative fifteen to twenty seconds at a distance of twelve inches from the light.

Metol-hydro developer:—

20 ounces water	600 cc.
30 grains metol	2 grammes
10 grains hydrochinon	$\frac{3}{4}$ gramme
110 grains sulphite soda gran.	7 grammes
110 grains carbonate soda gran.	7 grammes
2 grains potassium bromide	$\frac{1}{10}$ gramme

For soft-detail negatives, shorten exposure and use full strength; for vigorous negatives, dilute one-half with water. Lower the flame of lamp or gas to about one-half, take the exposed paper and draw through the developer face up, thus wetting front and back; if exposure is correct development will proceed uniformly, and be completed in thirty to sixty seconds. To arrest development place at once in following solution:—

Short stop and hardener:—

32 ounces water	960 cc.
1 ounce powdered alum	30 grammes
1 ounce table salt	30 grammes

Thirty to sixty seconds' immersion is sufficient; rinse and place in fixing-bath.

32 ounces water	960 cc.
1 drachm sulphuric acid	4 cc.
1 ounce sulphite of soda (dry)	30 grammes
8 ounces hyposulphite soda	240 grammes

Prints should remain in fixing solution not less than ten minutes, wash in running water for thirty minutes to one hour, or twelve to fifteen changes of water. After well rinsing the prints from hypo bath, if placed in the alum and salt bath for five minutes, it will both harden the surface and greatly tend to eliminate the hyposulphite of soda and shorten time of washing.

REMARKS. — To make prints *flexible*, after washing immerse for a few minutes in one to ten glycerin and water. Bromide in developer is only needed if negatives are flat and lack contrast, then a few drops of a 10 per cent solution potassium bromid can be added to developer.

CAUTION. — To secure success from the start with vinco hold the negative at least twelve inches from gas or lamp light, and if a Welsbach, fifteen to twenty is better. Also follow our instructions as to using a *weak* developer; overexposure and a strong developer are fatal to best effects with vinco.

To stop development locally. — It frequently happens that landscape negatives have delicate detail in the shadows; to preserve them in the print, as soon as the image is out far enough in the shadows, remove from developer, rub over the parts with a finger dipped in the *short stop*, or with a tuft of cotton wet with same. This will arrest development in those parts, while allowing the rest to proceed.

43. Velox developing-paper: —

0.5 gramme metol	7 grains
14.2 grammes sodium sulphite crystals	$\frac{1}{2}$ ounce
2 grammes hydrochinon	30 grains
28 grammes sodium carbonate crystals	400 grains
296 cc. water	10 ounces
10 drops bromide of potassium, 10 per cent	about 10 drops

Or,

118.4 cc. water	4 ounces
14 grammes sodium sulphite crystals	200 grains
1.4 grammes amidol	20 grains
5 drops 10 per cent bromide of potassium solution	about 5 drops

If blacks are greenish, add more amidol; if whites are grayish, add more bromide of potassium.

44. Fixing-bath:—

453.6 grammes hyposulphite of soda	16 ounces
1894 cc. water	64 ounces

Then add the following hardening solution:—

148 cc. water	5 ounces
14 grammes sodium sulphite crystals	$\frac{1}{2}$ ounce
88.8 cc. commercial acetic acid (25 per cent pure acid)	3 ounces
14 grammes powdered alum	$\frac{1}{2}$ ounce

45. Blue-prints:—

No. 1

53.2 grammes citrate of iron and ammonia . . .	1 $\frac{7}{8}$ ounces
236.8 cc. water	8 ounces

No. 2

35.5 grammes ferricyanid of potassium	1 $\frac{1}{4}$ ounces
236.8 cc. water	8 ounces

Mix equal parts of No. 1 and No. 2, and float the paper on the solution for three minutes. Plain Rives paper should be used. Hang up to dry in dark room. (*International Annual*, 1902.)

Prolonged washing will clear the lights.

Overexposure may be corrected by washing in water containing a very small amount of ammonia—10 drops of strong ammonia to 1 pint of water—and gradually increasing the strength until the lights are cleared. Dilute solution of oxalic acid will work the same results.

The colors may be brightened by washing in water with a trace of hydrochloric acid or citric acid.

Culture Methods

46. Method for making the three principal media based on the recommendation of the Bacteriological Committee to the American Public Health Association. Modified by H. W. Hill, Boston Board of Health. (*Jour. App. Mic.*, Vol. II, p. 301.)

Boil 30 grammes thread agar in 1 liter of water for half an hour. Make up loss by evaporation to a weight of 1000 grammes. Cool and solidify.

Nutrient Broth	Nutrient Gelatin	Nutrient Agar
1. Infuse lean meat twenty hours with twice its weight of distilled water in refrigerator, say 1000 grammes meat, 2000 grammes water.	Ditto.	Infuse lean meat twenty hours with <i>its own</i> weight of distilled water in refrigerator, say 1000 grammes meat, 1000 grammes water.
2. Make up weight of meat infusion (and meat) to original weight by adding water, <i>i.e.</i> to 3000 grammes.	Ditto.	Ditto, <i>i.e.</i> to 2000 grammes.
3. Filter infusion through cloth to remove meat.	Ditto.	Ditto.
4. Titrate and record reaction of filtrate, say reaction + 2.2 per cent.	Ditto.	Ditto, say reaction + 4.2 per cent.
5. Weigh infusion, say 1800 grammes.	Ditto.	Ditto, say 900 grammes.
6. Set infusion on water-bath, keeping temperature below 60°C.	Ditto.	Ditto.
7. Add peptone, 1 per cent, 18 grammes; add salt, 0.5 per cent, 9 grammes.	Ditto, and sheet gelatin, 10 per cent, 180 grammes.	Add peptone, 2 per cent, 18 grammes; add salt, 1 per cent, 9 grammes.
8. After ingredients are titrated, reaction probably + 2.3 to + 2.5.	Ditto; reaction probably + 4.0 to + 5.0.	Ditto; reaction probably + 4.5 to + 4.7.
9. Neutralize (Fuller's method).	Ditto.	Ditto.

To the 900 grammes of meat infusion (containing now peptone and salt also) add 900 grammes of the 3 per cent agar jelly described at head of this column. Since agar is neutral, reaction is unchanged.

10. Heat over boiling water (or steam) bath thirty minutes.
11. Restore weight lost by evaporation to original weight of filtered meat infusion, *i.e.* that on which the percentage of peptone, salt, etc., were calculated, 1800 grammes in *each case*.
12. Titrate, reaction probably $+0.3$ to $+0.5$.
13. Adjust reaction to final point desired, generally to $+1.5$ per cent.
14. Boil five minutes over free flame, with stirring.
15. Add water if necessary to make up loss from evaporation to 1800 grammes.
16. Filter through absorbent cotton, passing the filtrate through the filter repeatedly until clear.
17. Titrate to determine whether or not the desired reaction has been maintained.
18. Tube and sterilize.

47. James H. Wright's method of anaërobic cultivation in fluid media. (*Four. App. Mic.*, Vol. III, p. 1006.)

The adjoining figures (122 and 123) represent a simple apparatus used successfully by the author in the cultivation of tetanus bacillus. The apparatus consists of a simple arrangement of glass and rubber tubes enclosed in an ordinary test-tube, with a plug of cotton inserted in its mouth, as in an ordinary culture tube.

When it is desired to make an anaërobic culture, the culture fluid is put in the test-tube, and inoculated in the usual way. By suction the fluid is drawn up into the system of tubes to a level above *C*, after which the rubber tube *E* is compressed with the fingers, and the tube *D* pushed downward into the test-tube in such a manner as to fold the rubber tubes *D* and *C*, thus rendering the fluid *A* perfectly air-tight.

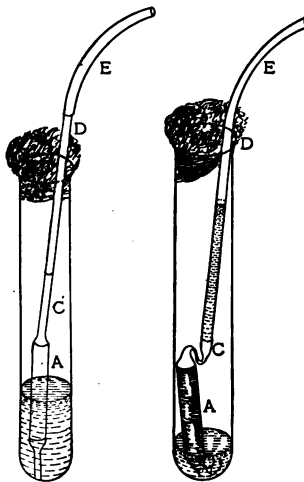


FIG. 122.

FIG. 123.

Wright's Anaërobic Apparatus.

48. Preparation of potato for culture of bacteria :—

Select several sound potatoes, and boil them for about twenty or thirty minutes in a vessel of water. Pour out the water, and allow the potatoes to cool. Divide them with a sterilized knife. The inoculation is performed in the usual way, after being assured that the potato is thoroughly sterilized before the inoculation is done.

Stains and Staining

49. Beale's carmine :—

Carmine 0.6 gramme

Dissolved in boiling solution of ammonia. Let stand for an hour to cool and to permit superfluous ammonia to escape. Add to the solution :—

Water 60 cc.
Glycerin 60 grammes
Absolute alcohol 15 grammes

Permit to stand for some time and filter.

50. Anilin violet (Hanstein's "Practical Botany," Bower, p. 331): "Dissolve equal parts of fuchsin and methyl-violet in alcohol. It stains cellulose cell walls of a faint violet color, and lignified cell walls deep violet. It is especially useful for bringing out the different parts of the bast, since the bast-fibres stain red, whereas the sieve-tubes and the parenchyma scarcely stain at all. The protoplasm is stained pink; amyloid substances, gums, and nuclei stain different shades of red; resins, blue; and tannin, brick-red."

51. Differential nucleolar staining (Gustave Mann. See *Jour. Roy. Mic. Soc.*, p. 690, 1891.): "Tissues, both vegetable and animal, preferably fixed by picro-corrosive method (Mann's), are treated for ten minutes in a saturated solution of heliocin in 50 per cent alcohol; the sections are then transferred for from five to fifteen minutes to a standard watery solution of anilin blue. The superfluous stain is rapidly washed off by distilled water, and the sections placed again for one or two

minutes in the heliocin solution, dehydrated, cleared by resinified turpentine, and mounted in turpentine balsam. The whole of the cell and the nucleus are stained blue, the nucleolus red."

52. Staining of Chlorophyl (Mann. See *Jour. Roy. Mic. Soc.*, p. 689, 1891.): "A glass vessel is filled with two liters of water, to which six drops are added of a 10 per cent solution of cyanin in absolute alcohol. Then a small quantity of either *Spirogyra jugalis* or *Spirogyra nitida* is placed in the vessel, which is exposed to the bright daylight. After some time, varying with the temperature of the room and the activity of the threads, from three to twenty-four hours, the whole of the cyanin will have been taken up by the threads. The ground-substance of the chlorophyl bands will have changed from a green to a bluish green color, while the oil globules and many of the microsomes between the bands will have turned blue, showing their fatty nature."

53. Staining cell nuclei of pollen-grains (Herr A. Meyer's method. See *Jour. Roy. Mic. Soc.*, p. 899, 1892.):—

Carmine	0.5 gramme
Absolute alcohol	20 ccm.
Hydrochloric acid	30 drops
Heat for thirty minutes in water-bath and add	
Chloral hydrate	25 grammes

Filter and cool. Stains nuclei of pollen-grains within ten minutes an intense red.

54. Staining paraffin sections (*Amer. Mic. Jour.*, XI, p. 11, 1890): Dissolve the coloring matter in absolute alcohol, and drop the solution in turpentine until the desired depth is secured. Sections fixed to the slip by the collodion method are placed in the oven until the oil of cloves is completely evaporated; the paraffin is dissolved in turpentine, and the slide is then put in the stain. The action is quick. Overstaining may be corrected by placing in a solution made with equal parts of acid, free absolute alcohol, and turpentine. Meyer's carmine, methyl-green, methyl-blue, gentian violet, safranin, Bismarck brown, eosin fuchsin, may be used as indicated with good results.

55. Staining of protoplasts and cell walls (Herr J. af Klercher, *Jour. Roy. Mic. Soc.*, p. 562, 1893, and *Verhandl. Biol. Ver. Stockholm*, IV, No. 14, 1892): "If the object examined is an aërial part of a plant, the oily substances are first removed by ether or dilute ammonia, and, after washing out the fixing material, the object is allowed somewhat to dry in order to promote the entrance of the staining substance. But if it is only the membrane which is to be stained, the object is brought directly, or after washing out the fixing material, into eau de Javelle or eau de Labarracque, and left there till all the protoplasm is dissolved. After careful washing, it is then stained with a moderately concentrated solution of Congo red, and finally, after careful washing, placed in paraffin. A good staining of membranes may also be effected by successive treatment with iron salts and potassium ferrocyanid, or with tannin and ferric chlorid."

56. Alum-carminé (Boneval. See *Amer. Mic. Jour.*, p. 78, 1894.):—

Ammonia alum	1 to 5 grammes
Carminé	4 grammes
Distilled water	100 grammes

"Boil for twenty minutes, taking care to maintain the original volume by adding water. Filter and preserve by a crystal of thymol. It is a nuclear stain of the first order; it colors admirably the nuclei of tissues fixed by osmic acid, which makes it valuable in many instances where picro-carminé is worthless. The color is well preserved in glycerin. Place the sections for a few hours in a vessel containing one cc. of alum-carminé, and wash until the excess of color is removed. This stain is exceedingly penetrating, so that tissues may be colored *en masse*."

57. Rosanilin-violet (Hanstein's "Practical Botany," Strasburger, p. 398): "Equal parts of methyl-violet and fuchsin (majenta), mixed and dissolved in alcohol. Shows stratification of cell walls, and differentiates sections of stems, especially monocotyledons. Stains protoplasm bluish violet; amyloid sub-

stances, nucleus, and gums, different shades of red; resins, blue; tannin, foxy red; cellulose, pale violet; lignin, reddish; bast-fibres, deep red; sieve-tubes and bast parenchyma, hardly at all."

58. Gram's method for staining Diphtheria Bacilli: Allow the fixed specimens to remain for 20 to 30 minutes in an anilin-water solution of gentian violet, prepared in the following manner:—

To 100 cc. of distilled water add, drop by drop, anilin oil until the mixture is opaque. Shake well after each addition of anilin oil. Filter through moistened filter-paper until perfectly clear. To 100 cc. of the filtrate add 10 cc. of absolute alcohol and 11 cc. of concentrated alcoholic solution of gentian violet.

After remaining the required time in this mixture the specimens are placed for about five minutes in the following iodine solution:—

Iodin	1 gramme
Potassium iodid	2 grammes
Distilled water	300 cc.

This solution should be allowed to act until the specimens are black, after which they are thoroughly washed in alcohol, which removes the black color, causing the specimens to appear pale gray. Dry and mount in balsam, or contrast stain with carmin or Bismarck brown. (*Jour. App. Mic.*, Vol. IV, p. 1476.)

59. To remove anilin stain from the fingers:—

a. Use successively a 5 per cent solution of sodium chlorid, feroxid of hydrogen, and lastly alcohol. (*Jour. Mic. and Nat. Sci.*, Vol. II, p. 157.)

b. To remove silver stains from fingers:—

14.2 grammes sulphate of sodium	$\frac{1}{2}$ ounce
7 grammes chlorid of lime	$\frac{1}{2}$ ounce
29.6 cc. water	1 ounce

c. To remove pyrogallie acid from hands. Wash with a 10 per cent solution of oxalic acid, or sulphuric acid diluted with water 1:20.

d. To remove developer stains from fingers, use lemon juice.

Miscellaneous Recipes

60. Dead black:—

Ivory or lampblack 2 grains, a drop or two of gold size, thoroughly mix and add 24 drops of spirits of turpentine. Apply with camel's-hair brush to produce a fine coating of black.

61. Black polish on brass:—

1.	28.4 grammes	nitrate of silver	.	.	.	.	1 ounce
	592 cc.	water	.	.	.	.	20 ounces
2.	28.4 grammes	nitrate of copper	.	.	.	.	1 ounce
	592 cc.	water	.	.	.	.	20 ounces

Mix the two solutions together and dip the brass into it and heat in an oven until the degree of black is secured. (*National Druggist*.)

Another recipe for blackening brass: Clean from all grease, cover with a solution of nitrate of copper and apply heat. May be lacquered by applying shellac varnish and gently heating.

62. Dead-black surface on brass: To 2 grains of lampblack add just enough gold size as will hold the lampblack and mix well. Add drop by drop, and when well mixed add 24 drops of turpentine and stir thoroughly. Apply with camel's-hair brush. (L. A. WILSON, *The Mic.*, 1897.)

63. Blackening iron and steel: Clean thoroughly and immerse in

Mercuric chlorid	.	.	.	.	.	.	4 parts
Cupric chlorid	.	.	.	.	.	.	2 parts
Hydrochloric acid	.	.	.	.	.	.	12 parts
Alcohol	.	.	.	.	.	.	10 parts
Water	.	.	.	.	.	.	100 parts

Dry and place for half an hour in boiling water. If color is not deep enough, repeat the operation. (*Revue Suisse*.)

64. To keep metallic objects from rusting:—

White wax	.	.	.	.	.	.	11 parts
Suet	.	.	.	.	.	.	2 parts

Dissolve in spirits of turpentine.

65. Ink for writing on glass : —

Brown shellac	20 grammes
Alcohol	150 cc.

Dissolve and then add drop by drop a solution of

Borax	35 grammes
Distilled water	250 cc.

If this precipitates the shellac, add more alcohol and allow the excess to evaporate. One gramme of methyl-blue will give the color. (*Zeit. f. angew. Mikr.*, Bd. V.)

66. Etching fluid for glass : —

1. Dissolve sodium fluorite 36 grammes
in distilled water 500 cc.
and add potassium sulphate 7 grammes
2. Dissolve zinc chlorid 14 grammes
in distilled water 500 cc.
and add concentrated hydrochloric acid 65 grammes

These solutions can be kept in ordinary glass vessels. When wanted for use equal volumes are mixed in a hollow paraffin cube. A small quantity of India ink added will enable the writer to see what he is doing. (*Cent. Zeit. f. Opti. u. Mech.*, XII, p. 57.)

67. Another etching fluid: Mix 10 parts of fluorid of ammonia with 40 parts of sulphate of barium, and add sulphuric acid. The salts are previously well mixed in a leaden vessel. The following method is also good: equal parts of fluorid of ammonia and sulphate of barium are dissolved in fluoric acid. The writing is done with an ordinary steel pen. After half an hour the writing surface is washed with water. (Anthony's *Photo. Bull.*, Vol. XXXI, p. 120.)

68. Writing on glass : —

Shellac	2 parts
Venice turpentine	1 part
Oil of turpentine	3 parts
Lampblack	1 part

Dissolve in water-bath.

69. Melt together spermaceti four, tallow three, and wax two parts; and add six parts of either red lead, white lead, or Prussian blue, according to color desired. The mass is turned out in sticks for use. (*Popular Science News*, Formulæ or Faber's.)

70. The so-called diamond ink, for writing on glass, consists of a mixture of fluoric acid and barium. The latter is used as a body for the acid. Permit to remain on the glass for fifteen minutes and then rub off the barium, and the characters will be impressed in the glass.

71. The emission of pollen-tubes: Dr. B. D. Halsted recommends that pollen-grains be placed in a 10 to 70 per cent solution of sugar, and the pollen-grains will produce tubes within a few hours.

72. Absolute alcohol: Add anhydrous cupric sulphate to 95 per cent alcohol. The pulverized cupric sulphate is first heated to red heat to drive off the water of crystallization, and, after cooling, is shaken in the alcohol and the mixture allowed to stand a day, the liquid decanted, fresh cupric sulphate added, and the operation repeated until the blue color due to the presence of water is almost, if not quite, destroyed. To test, treat a drop of the alcohol with a drop of turpentine on a slide and examine under the microscope to detect particles of water.

73. Softening dried material:—

Caustic potash		1 part
Glycerin		5½ parts
Water		5 parts

74. Schultze's macerating fluid: This is used for separating the parts of woody tissue and is prepared by dissolving in 50 cc. of nitric acid 1 gramme of potassium chlorate. Used in small quantities, and metallic instruments must be protected from its effects.

75. To clean the hands of cements:—

Castile soap, shaved fine		15 parts
Alcohol, 95 per cent		10 parts
Benzol, ordinary		10 parts
Ammonia water		5 parts
Glycerin		5 parts

Dissolve the soap in the alcohol, add the ammonia and benzol, and after thorough agitation the glycerin. After using the soap, wash the hands and finish by rubbing over them a few drops of glycerin.

76. Solution for silvering glass (Burton's):—

1.	1.8 grammes nitrate of silver	25 grains
	29.6 cc. distilled water	1 ounce
2.	1.8 grammes potash C. P.	25 grains
	29.8 cc. distilled water	1 ounce

A

Equal parts of solution 1 and 2. Ammonia to just dissolve precipitate; solution 1 to just cause coloration.

B

189	grammes loaf sugar	2700 grains
592	cc. distilled water	20 ounces
7.4	cc. nitric acid	2 drachms
296	cc. alcohol (strong)	10 ounces
2368	cc. distilled water to make up to	80 ounces

For use:—

29.6 cc. Solution A	1 ounce
29.6 cc. Solution B	1 ounce

Solution B improves with age, while A deteriorates.

77. Silvering glass (by M. M. Auguste and Louis Lumière):
 "To 100 cc. of a 10 per cent solution of silver nitrate, ammonia is added drop by drop until the precipitate formed is redissolved. Too much ammonia must not be added at first, for this might prevent the formation of the precipitate. The volume of the solution is increased to a liter by the addition of distilled water. This is solution A. Solution B is made by diluting commercial formaldehyde of 40 per cent with distilled water so as to form a 1 per cent solution. Solution B can be kept for some time. Two volumes of A are rapidly mixed with one volume of B, and the mixture is rapidly poured over the glass to be coated. In five or six minutes, at a temperature of 15° to 19° C. all silver in

the solution is deposited in a brilliant layer, which can then be washed with water. (*Jour. de Physique*, January, 1895, and *Amer. Jour. Sci.* June, 1895.)

78. Silvering glass with formalin: Dissolve 1 gramme nitrate of silver in 100 cc. of distilled water, and carefully add ammonia until the precipitate has dissolved again. An excess of ammonia should be avoided as much as possible. Dilute the solution with distilled water to 1 liter. In a second bottle dilute 25 cc. formalin solution with distilled water to 1 liter. The glass plate to be silvered is carefully cleaned and waxed around the edges, and it is then laid in a horizontal position. Two parts of the silver solution are then mixed quickly with 1 part of the formalin solution, and the mixture poured upon the glass plate. After ten minutes the silver will have precipitated upon the glass plate, when the liquid may be poured off and the mirror is ready to be washed and cleaned under the faucet. (Anthony's *Photo. Bull.*, Vol. XXXI, p. 288.)

79. Ground-glass varnish:—

6.3 grammes sandarac	.	.	.	.	.	90 grains
1.4 grammes mastic	.	.	.	.	.	20 grains
59.2 cc.	ether	.	.	.	.	2 ounces
14.8 to 44.4 cc.	benzol	.	.	.	.	$\frac{1}{2}$ to $1\frac{1}{2}$ ounces

The proportion of the benzol determines the character of the surface.

80. To clean a negative stained with silver: Rub with cotton moistened with a solution of cyanid of potassium, wash well and dry.

81. Size of photographic glass and mounts:—

Petite	.	.	.	.	.	.	.	$1\frac{5}{8} \times 3\frac{1}{8}$ inches
One-ninth plate	.	.	.	.	.	.	.	$2 \times 2\frac{1}{2}$ inches
One-sixth plate	.	.	.	.	.	.	.	$2\frac{1}{2} \times 3\frac{1}{2}$ inches
One-fourth plate	.	.	.	.	.	.	.	$3\frac{1}{4} \times 4\frac{1}{4}$ inches
Half plate	.	.	.	.	.	.	.	$4\frac{1}{2} \times 5\frac{1}{2}$ and $4\frac{1}{2} \times 6\frac{1}{2}$ inches
Whole plate (4-4)	.	.	.	.	.	.	.	$6\frac{1}{2} \times 8\frac{1}{2}$ inches
Extra (4-4)	.	.	.	.	.	.	.	8×10 inches

82. Fixing microscopic objects to the glass slip. Warm the glass slip (40° to 50° C.) to remove all trace of moisture, apply a drop of mixture prepared as follows:—

White lac	15 grammes
Absolute alcohol	100 grammes

Dissolve in water-bath and decant off clear liquid.

After alcohol is evaporated from the slip, a hard transparent coating is left. This may be softened by a drop of oil of lavender. Arrange the object on the slip, heat over the lamp until the oil of lavender is evaporated and the object is left firmly attached. (M. J. TEMPÈRE, in *Micrographie Préparateur*.)

83. Wickersheim's solution for preserving specimens in large quantities:—

Alum	100 grammes
Saltpetre	12 grammes
Potash	60 grammes
Arsenious oxid	20 grammes
Boiled water	3000 grammes

84. Virodteff's solution for preserving large specimens:—

Glycerin	2160 grammes
Water	1080 cc.
Alcohol	45 cc.
Thymol	5 grammes

85. Kaiserling's method for preserving pathological specimens. It is claimed for this method that the natural colors of the specimens are preserved almost exact. Place in the following solution, for 3 to 5 days:—

No. 1

Formalin	40 parts
Water	200 parts
Potassium nitrate	3 parts
Potassium acetate	6 parts

The specimens must not be washed out in running water, as it removes the blood on which the color depends. An excess of blood should be wiped off before placing the specimens in No. 1. Specimens lose their color after remaining in solution for a few days, but the color soon returns after the specimens are transferred to

No. 2

Alcohol	4 parts
Water	1 part

in which they remain for 3 to 5 hours, after which they are placed in

No. 3

Alcohol, 95 per cent,

for 1 to 2 hours, and finally are permanently preserved in

No. 4

Potassium acetate	1 part
Glycerin	2 parts
Water (distilled)	10 parts

which, before use, should be allowed to stand for 48 hours and then filtered. Keep the specimens in a dark place, because the light fades the color in course of time. (*Texas Med. News.*, Vol. X, p. 10.)

86. Marlmann's method for preserving the colors of anatomical specimens, and for fixing and preserving specimens where it is desirable to avoid alcohol.

No. 1

Sodium fluorid	50 grammes
Formaldehyd (40 per cent)	20 cc.
Water	1000 cc.

From this fluid the preparations are passed into the following mixture for preservation: —

Glycerin (28° B)		500 cc.
Water		1000 cc.
Magnesium chlorid		100 grammes
Sodium fluorid		20 grammes

Objects for sections should be washed three or four times in water, and then treated with the usual grades of alcohol. (*Jour. App. Mic.*, Vol. IV, p. 1582.)

87. Decalcifying solution : —

Nitric acid		3 parts
Chromic acid		70 parts
Water		200 parts

After bone becomes soft enough to permit a needle to enter, wash in water and harden in alcohol.

88. Another : —

- (1) Imbed in celloidin in the usual way.
- (2) Harden by slow evaporation in alcohol or chloroform.
- (3) The celloidin blocks are then immersed in mixture of alcohol 90 per cent and nitric acid; the proportion of latter is regulated by the amount of calcareous matter in specimen (10 to 100 of alcohol).
- (4) Renew decalcifying fluid from time to time.
- (5) Remove excess of acid by washing.
- (6) Immerse in alcohol 90 per cent, changed for several days.
- (7) Section the celloidin block after removing acid. (*Bull. Soc. Belge de Mic.*, XXIII, p. 159.)

89. Lacquering microscope tubes and stands: Cork the tube to prevent the ingress of the cleaning solutions and then wash in the following soap mixture to remove old lacquer : —

Shaved castile soap		1 part
Alcohol 95°		3 parts
Benzol		3-4 parts
Liquid potassæ		1 part

Polish with Putz pomade and finish with chamois skin sprinkled with finest levigated chalk. Wash a second time in soap mix-

ture. Place then in hot water, wipe dry, and apply either one of the following lacquers with a camel's-hair brush, with the strokes all in the same direction. The water must not be above 66° to 71° C., otherwise there is danger of blistering the lacquer.

(1) Pale gold lacquer : —

Shellac quite clean	1 part
Ground turmeric	1 part
Alcohol	4 parts

Mix and set aside in a warm place with frequent agitation until lacquer is dissolved. Decant clear liquid and preserve for use.

(2) Yellow gold : —

Shellac	25 parts
Turmeric	4 parts
Dragon's blood	1 part
Alcohol 95°	70 parts

Digest for eight days with frequent agitation; decant and filter.

(3) Deep-red gold : —

Spanish anatto	2 parts
Turmeric powdered	30 parts
Saunders's red	3 parts
Alcohol 95°	480 parts

Infuse in the cold for 24 to 36 hours, shaking occasionally. Let stand until settled and then add : —

Shellac	60 parts
Sanderac	15 parts
Mastic	15 parts
Canada balsam	15 parts

When dissolved, add 10 parts of oil of turpentine. Upon highly polished brass this gives a deep-red gold color that is lasting and very handsome. (F. L. JAMES.)

90. Paste for mounting botanical specimens :—

Tragacanth in powder	30 parts
Gum arabic	20 parts
Glycerin	30 parts
Water	60 parts
Bichlorid of mercury	1 part
Boiling water	240 parts

Mix the gums with glycerin and water in a mortar with vigorous stirring. Dissolve the bichlorid of mercury in boiling water and add to the gum mixture. When cold put in a few drops of oil of cloves. (*Nat. Drug.*, p. 209, 1889.)

91. Cement for joining parts of apparatus, which will resist heat, water, acids, and oils.

Mix concentrated glycerin with finely powdered litharge to a thick paste. Glass, metal, and wood can be cemented together by it. (*Jour. Mic. and Nat. Sci.*, Vol. II, p. 53.)

92. Another: Gum elastic dissolved in benzin until liquid has syrupy consistency. White lead and linseed oil varnish are rubbed up to a paste and mixed with the gum. Cements glass to glass or wood to glass. Good for aquarium. (*Roy. Mic. Jour.*)

93. Apparatus for removing air bubbles from slide in mounting microscopical objects: Consists of a slip of plate glass (Fig.

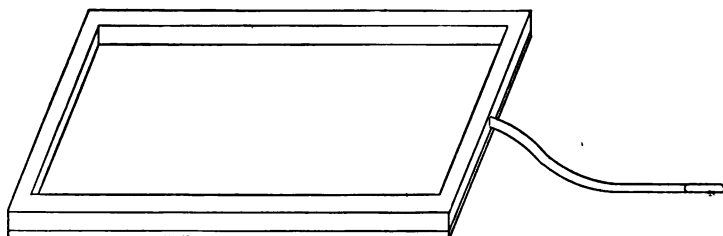


FIG. 124. — Apparatus for removing Air Bubbles from Slide.

124) measuring $4'' \times 1\frac{1}{2}''$, to which a frame of wood has been cemented. The frame is of such a size as to allow the ordinary glass micro slips being placed on it. In one side of the frame

is a hole bored, and one end of a piece of india-rubber tube measuring 6 inches long by $\frac{3}{16}$ inch in diameter is cemented to it. A piece of glass tubing *B* 1 inch in length is closed at one end; and a small hole is bored through it at about a quarter of an inch from the closed end. The open end of the glass tube is then slipped into the free end of the rubber tube, and the two so arranged that the hole in the former shall be just covered by the india-rubber. To use the apparatus, place the mount containing the air bubbles in the wooden cell and cover it with a second slip of glass, the edges of which have been previously greased with tallow. Exhaust the air by drawing through the tube. The valve formed by the hole will prevent the reëtrance of the air, and any bubbles in the mount will quickly disappear. (J. H. COOKE, in *Jour. App. Mic.*, Vol. II, p. 621.)

94. Cutting glass tubes and bottles: Cut strips of blotting-paper from $\frac{1}{4}$ to $\frac{1}{2}$ inch in width, wet and wrap around the bottle $\frac{1}{4}$ to $\frac{3}{8}$ inch apart. Throw a fine flame 2 or 3 inches long between the wet papers, revolving the bottle slowly. Within a minute the glass will break with a clear cut along the line followed by the flame. (PROFESSOR WILLIAM THOMPSON, *Jour. Mic. and Nat. Sci.*)

95. Paste for labels:—

Gum arabic	.	.	.	.	.	.	.	.	15.0 parts
Tragacanth	.	.	.	.	.	.	.	.	7.5 parts
Glycerin	.	.	.	.	.	.	.	.	45.0 parts
Thymol	.	.	.	.	.	.	.	.	0.3 part
Alcohol	.	.	.	.	.	.	.	.	3.75 parts
Water to make up to	.	.	.	.	.	.	.	.	120.0 parts

Dissolve gum arabic in 15 parts of water, rub up tragacanth in 30 parts of water. Then mix and strain. Add the glycerin and finally the alcohol and thymol. (*Zeit. f. Angew. Mikr.*, III.)

96. Loosen stoppers by placing a little glycerin around the stopper; and smearing some glycerin on the portion of stop-

per going into the bottle will prevent it from becoming tightened.

97. Refractive indexes and alcohol solvent powers of clearing and mounting media. (E. M. BRACE, in *Jour. App. Mic.*, Vol. I, p. 220.)

Substances	Refractive Index	Per cent of Alcohol which Substance will dissolve
Cloroform	1.459	80 %
Oil of Cedar	1.466	95 %
Turpentine	1.469	100 %
Oil of Orange	1.472	95 %
Oil of Origanum	1.479	95 %
Xylene	1.493	95 %
Benzin	1.495	95 %
Oil of Thyme	1.496	95 %
Oil of Cedarwood	1.502	95 %
Xylene Balsam	1.524	—
Oil of Cloves	1.532	80 %
Oil of Anilin	1.585	60 %
Oil of Cassia	1.602	80 %

98. Retouching formula for negatives: Equal parts of gold and benzol make an excellent cold varnish for negatives, which gives a surface ready for the retouching pencil without further preparation. (Wilson's *Photo. Mag.*, December, 1901.)

99. To title negatives:—

A. Sugar	1 part
Glycerin	3 parts
Water	10 parts
B. Mercuric chlorid	1 part
Mercuric nitrate	2 parts
Alcohol	10 parts

Mix equal parts of A and B and write on paper. Transfer the writing to the film by pressing it into close contact. (Wilson's *Photo. Mag.*, Vol. III.)

100. A black finish for table-tops:—

No. 1. Copper sulphate	125 grammes
Potassium chlorate	125 grammes
Water	1000 grammes

Boil until dissolved.

No. 2. Anilin hydrochlorate	150 grammes
Water	1000 grammes
or	
Anilin oil	120 grammes
Hydrochloric acid	180 grammes
Water	1000 grammes

Apply to the table-top two coats of No. 1 while hot, and as soon as this first coat is dry apply a second coat. Then put on two coats of No. 2 and allow to dry. Rub in with a cloth a thin coating of linseed oil; rapid and lengthy rubbing in of the oil will give the polish to the wood. Wash out the surplus chemicals with hot soap-suds. The finish left is a deep ebony black. It is claimed that acids will not stain this table finish and that anilin stains can be washed off by alcohol. (*Bot. Zeit.*, 54, 326, and *Bot. Gaz.*, 24, 66.)

101. Another table-top finish:—

No. 1. Sodium chlorate	67 grammes
Copper chlorate	67 grammes
Water	1 liter
No. 2. Anilin chlorate	150 grammes
Water	1 liter

Paint the table with No. 1 and follow with No. 2 and allow to dry. Three applications will give a dense black. After the surface is dry, it can be finished with oil. (J. R. SLONAKER.)

102.—CONVERSION OF BRITISH AND METRIC MEASURES

Computed by Mr. E. M. Nelson from the New Coefficient obtained by order of the Board of Trade in 1896. (*Jour. Roy. Mic. Soc.*)

LINEAL

METRIC INTO BRITISH				BRITISH INTO METRIC			
μ	in.	mm.	in.	mm.	in.	mm.	in.
1	.000039	1	.039370	41	1.614175	1	25.399978
2	.000079	2	.078740	42	1.653545	2	50.799956
3	.000118	3	.118110	43	1.692915	3	76.199934
4	.000157	4	.157480	44	1.732285	4	101.599912
5	.000197	5	.196851	45	1.771655	5	126.999890
6	.000236	6	.236221	46	1.811025	6	152.399868
7	.000276	7	.275591	47	1.850395	7	177.799846
8	.000315	8	.314961	48	1.889765	8	203.199824
9	.000354	9	.354331	49	1.929136	9	228.599802
10	.000394	10	.393701	50	1.968506	10	253.999780
11	.000433	11	.433071	51	2.007876	11	279.399758
12	.000472	12	.472441	52	2.047246	1 ft.	304.799736
13	.000512	13	.511811	53	2.086616	1 yd.	914.399208
14	.000551	14	.551182	54	2.125986	in.	mm.
15	.000591	15	.590552	55	2.165356	1	25.399978
16	.000630	16	.629922	56	2.204726	2	50.799956
17	.000669	17	.669292	57	2.244096	3	76.199934
18	.000709	18	.708662	58	2.283467	4	101.599912
19	.000748	19	.748032	59	2.322837	5	126.999890
20	.000787	20	.787402	60	2.362207	6	152.399868
21	.000827	21	.826772	61	2.401577	7	177.799846
22	.000866	22	.866142	62	2.440947	8	203.199824
23	.000906	23	.905513	63	2.480317	9	228.599802
24	.000945	24	.944883	64	2.519687	10	253.999780
25	.000984	25	.984253	65	2.559057	11	279.399758
26	.001024	26	1.023623	66	2.598427	1 ft.	304.799736
27	.001063	27	1.062993	67	2.637798	1 yd.	914.399208
28	.001102	28	1.102363	68	2.677168	in.	mm.
29	.001142	29	1.141733	69	2.716538	1	25.399978
30	.001181	30	1.181103	70	2.755908	2	50.799956
31	.001220	31	1.220473	71	2.795278	3	76.199934
32	.001260	32	1.259844	72	2.834648	4	101.599912
33	.001299	33	1.299214	73	2.874018	5	126.999890
34	.001339	34	1.338584	74	2.913388	6	152.399868
35	.001378	35	1.377954	75	2.952758	7	177.799846
36	.001417	36	1.417324	76	2.992129	8	203.199824
37	.001457	37	1.456694	77	3.031499	9	228.599802
38	.001496	38	1.496064	78	3.070869	10	253.999780
39	.001535	39	1.535434	79	3.110239	11	279.399758
40	.001575	40	1.574805	80	3.149609	1 ft.	304.799736
						1 yd.	914.399208
						in.	mm.
						1	25.399978
						2	50.799956
						3	76.199934
						4	101.599912
						5	126.999890
						6	152.399868
						7	177.799846
						8	203.199824
						9	228.599802
						10	253.999780
						11	279.399758
						1 ft.	304.799736
						1 yd.	914.399208
						in.	mm.
						1	25.399978
						2	50.799956
						3	76.199934
						4	101.599912
						5	126.999890
						6	152.399868
						7	177.799846
						8	203.199824
						9	228.599802
						10	253.999780
						11	279.399758
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						in.	mm.
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						11	279.399758
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						in.	mm.
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						in.	mm.
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						5	126.999890
						6	152.399868
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						5	126.999890
						6	152.399868
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						in.	mm.
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						3	76.199934
						4	101.599912
						5	126.999890
						6	152.399868
						7	177.799846
						8	203.199824
						9	228.599802
						10	253.999780
						11	279.399758
						1 ft.	304.799736
						1 yd.	914.399208
						in.	mm.
						1	25.399978
						2	50.799956
						3	76.199934

CONVERSION OF BRITISH AND METRIC MEASURES CONTINUED

LINEAL

METRIC INTO BRITISH				BRITISH INTO METRIC					
μ	in.	mm.	in.	mm.	in.	mm.	in.		
41	.001614	81	3.188979	96	3.779531	$\frac{1}{8}$ s	.390769	$\frac{1}{7}$ s	.033867
42	.001654	82	3.228349	97	3.818901	$\frac{1}{7}$ s	.362857	$\frac{1}{8}$ s	.031750
43	.001693	83	3.267719	98	3.858271	$\frac{1}{6}$ s	.338666	$\frac{1}{7}$ s	.029882
44	.001732	84	3.307089	99	3.897641	$\frac{1}{5}$ s	.317500	$\frac{1}{8}$ s	.028222
45	.001772	85	3.346460			$\frac{1}{4}$ s	.298823	$\frac{1}{7}$ s	.026737
46	.001811	86	3.385830	dm.	in.	$\frac{1}{3}$ s	.282222		
47	.001850	87	3.425200	1	3.9370113	$\frac{1}{2}$ s	.267368	in.	μ
48	.001890	88	3.464570	2	7.8740226	$\frac{1}{4}$ s	.254000	$\frac{1}{10}$ s	25.399978
49	.001929	89	3.503940	3	11.8110339	$\frac{1}{3}$ s	.169333	$\frac{1}{20}$ s	12.699989
50	.001969	90	3.543310	4	15.7480452	$\frac{1}{2}$ s	.127000	$\frac{1}{30}$ s	8.466659
60	.002362	91	3.582680	5	19.6850565	$\frac{1}{3}$ s	.101600	$\frac{1}{40}$ s	6.349994
70	.002756	92	3.622050	6	23.6220678	$\frac{1}{2}$ s	.084667	$\frac{1}{50}$ s	5.079996
80	.003150	93	3.661420	7	27.5590791	$\frac{1}{3}$ s	.072571	$\frac{1}{60}$ s	4.233330
90	.003543	94	3.700791	8	31.4960904	$\frac{1}{2}$ s	.063500	$\frac{1}{70}$ s	3.628568
100	.003937	95	3.740161	9	35.4331017	$\frac{1}{3}$ s	.056444	$\frac{1}{80}$ s	3.174997
200	.007874					$\frac{1}{2}$ s	.050800	$\frac{1}{90}$ s	2.822220
300	.011811					$\frac{1}{2}$ s	.046182	$\frac{1}{100}$ s	2.539998
400	.015748					$\frac{1}{2}$ s	.042333	$\frac{1}{120}$ s	1.693332
500	.019685					$\frac{1}{2}$ s	.039077	$\frac{1}{140}$ s	1.269999
600	.023622					$\frac{1}{2}$ s	.036286	$\frac{1}{160}$ s	1.015999
700	.027559								
800	.031496								
900	.035433								
1000=(1 mm.)									
			1 meter = 3.2808428 ft.						
			= 1.09361426 yd.						

103.—METRIC SYSTEM OF WEIGHTS AND MEASURES

MEASURES OF LENGTH

DENOMINATIONS AND VALUES		EQUIVALENTS IN USE	
Myriameter . . .	10,000 meters	6.2137	miles
Kilometer . . .	1,000 meters	.62137	mile, or 3,280 ft. 10 in.
Hectometer . . .	100 meters	328.	feet and 1 inch
Dekameter . . .	10 meters	393.7	inches
Meter	1 meter	39.37	inches
Decimeter . . .	1-10th of a meter	3.937	inches
Centimeter . . .	1-100th of a meter	.3937	inch
Millimeter . . .	1-1000th of a meter	.0394	inch

MEASURES OF SURFACE

DENOMINATIONS AND VALUES		EQUIVALENTS IN USE	
Hectare	10,000 square meters	2.471	acres
Are	100 square meters	119.6	square yards
Centare	1 square meter	1,550.	square inches

MEASURES OF VOLUME

DENOMINATIONS AND VALUES			EQUIVALENTS IN USE	
NAMES	NO. OF LITERS	CUBIC MEASURES	DRY MEASURE	WINE MEASURE
Kiloliter or stere .	1,000	1 cm.	1.308 cubic yards	264.17 gallons
Hectoliter	100	1-10th cm.	2. bu. and 3.35 pecks	26.417 gallons
Dekaliter	10	10 cd.	9.08 quarts	2.6417 gallons
Liter	1	1 cd.	.908 quart	1.0567 quarts
Deciliter	1-10	1-10th cd.	6.1023 cubic inches	.845 gill
Centiliter	1-100	10 cc.	.6102 cubic inch	.338 fluid oz.
Milliliter	1-1000	1 cc.	.061 cubic inch	.27 fl. drin.

WEIGHTS

DENOMINATIONS AND VALUES			EQUIVALENTS IN USE	
NAMES	NUMBER OF GRAMS	WEIGHT OF VOLUME OF WATER AT ITS MAXIMUM DENSITY	AVOIRDUPOIS WEIGHT	
Millier or Tonneau . . .	1,000,000	1 cm.	2204.6	pounds
Quintal	100,000	1 hl.	220.46	pounds
Myriagram	10,000	10 l.	22.046	pounds
Kilogram or Kilo	1,000	1 l.	2.2046	pounds
Hectogram	100	1 dl.	3.5274	ounces
Dekagram	10	10 cc.	.3527	ounce
Gram	1	1 cc.	15.432	grains
Decigram	1-10	1-10th of a cc.	1.5432	grains
Centigram	1-100	10 cmm.	.1543	grain
Milligram	1-1000	1 cmm.	.0154	grain

104. — UNITED STATES WEIGHTS AND MEASURES

LINEAL

	Inches	Feet	Yards	Rods	Fur.	Mile
12 inches = 1 foot.	12 =	1				
3 feet = 1 yard.	36 =	3 =	1			
5.5 yards = 1 rod.	198 =	16.5 =	5.5 =	1		
40 rods = 1 furlong.	7,920 =	660 =	220 =	40 =	1	
8 furlongs = 1 mile.	63,360 =	5,280 =	1,760 =	320 =	8 =	1

SURFACE — LAND

	Feet	Yards	Rods	Roods	Acres
144 sq. in. = 1 sq. ft.					
9 sq. ft. = 1 sq. yard.	9 =	1			
30.25 sq. yd. = 1 sq. rod.	272.25 =	30.25 =	1		
40 sq. rods = 1 sq. rood.	10,890 =	1,210 =	40 =	1	
4 sq. roods = 1 acre.	43,560 =	4,849 =	160 =	4 =	1
640 acres = 1 sq. mile.	27,878,400 =	3,097,600 =	102,400 =	2,560 =	640

VOLUME — LIQUID

	Gills	Pints	Gallon	Cubic Inches
4 gills = 1 pint.				
2 pints = 1 quart.	32 =	8 =	1 =	231
4 quarts = 1 gallon.				

FLUID

Gallon	Pints.	Ounces	Drachms	Minims	Cubic Centimeters
1 =	8 =	128 =	1,024 =	61,440 =	3,785.435
	1 =	16 =	128 =	7,680 =	473.179
		1 =	8 =	480 =	29.574
			1 =	60 =	3.697

16 ounces, or a pint, is sometimes called a fluid pound.

TROY WEIGHT

Pound	Ounces	Pennyweights	Grains	Grams
1 =	12 =	240 =	5,760 =	373.24
	1 =	20 =	480 =	31.10
		1 =	24 =	1.56

APOTHECARIES' WEIGHT

lb		3		3		9		gr.		Grams
Pound		Ounces		Drachms		Scruples		Grains		
1	=	12	=	96	=	288	=	5,760	=	373.24
		1	=	8	=	24	=	480	=	31.10
				1	=	3	=	60	=	3.89
						1	=	20	=	1.30
								1	=	.07

The pound, ounce, and grain are the same as in Troy weight.
All chemicals are usually sold by

AVOIRDUPOIS WEIGHT

Pound		Ounces		Drachms		Grains (Troy)		Grams
1	=	16	=	256	=	7,000	=	453.60
		1	=	16	=	437.5	=	28.35
				1	=	27.34	=	1.77
						1	=	.07

UNITED STATES FLUID MEASURE

Gal.	Pints	Ounces	Drachms	Minims	Cu. In.	Grains	Cc.
1	= 8	= 128	= 1,024	= 61,440	= 231.	= 58,328.886	= 3,785.44
	1	= 16	= 128	= 7,680	= 28.875	= 7,291.1107	= 473.18
		1	= 8	= 480	= 18.047	= 455.6944	= 29.57
			1	= 60	= 0.2256	= 56.9618	= 3.70

IMPERIAL BRITISH FLUID MEASURE

Gal.	Pints	Ounces	Drachms	Minims	Cu. In.	Grains	Cc.
1	= 8	= 160	= 1,280	= 76,800	= 277.27384	= 70,000	= 4,543.732
	1	= 20	= 160	= 9,600	= 34.65923	= 8,750	= 567.966
		1	= 8	= 480	= 1.73296	= 437.5	= 28.398
			1	= 60	= 0.21662	= 54.69	= 3.550

105.—TABLE SHOWING CORRESPONDING DEGREES ON THE SCALE OF THE FAHRENHEIT AND CENTIGRADE THERMOMETERS

FAHR.	CENT.	FAHR.	CENT.	FAHR.	CENT.	FAHR.	CENT.	FAHR.	CENT.	FAHR.	CENT.
32.	0.	62.	16.6	91.4	33.	121.	49.4	152.	66.6	182.	83.3
33.	.5	62.6	17.	92.	33.3	122.	50.	152.6	67.	183.	83.8
33.8	1.	63.	17.2	93.	33.8	123.	50.5	153.	67.2	183.2	84.
34.	1.1	64.	17.7	93.2	34.	123.8	51.	154.	67.7	184.	84.4
35.	1.6	64.4	18.	94.	34.4	124.	51.1	154.4	68.	185.	85.
35.6	2.	65.	18.3	95.	35.	125.	51.6	155.	68.3	186.	85.5
36.	2.2	66.	18.8	96.	35.5	125.6	52.	156.	68.8	186.8	86.
37.	2.7	66.2	19.	96.8	36.	126.	52.2	156.2	69.	187.	86.1
37.4	3.	67.	19.4	97.	36.1	127.	52.7	157.	69.4	188.	86.6
38.	3.3	68.	20.	98.	36.6	127.4	53.	158.	70.	188.6	87.
39.	3.8	69.	20.5	98.6	37.	128.	53.3	159.	70.5	189.	87.2
39.2	4.	69.8	21.	99.	37.2	129.	53.8	159.8	71.	190.	87.7
40.	4.4	70.	21.1	100.	37.7	129.2	54.	160.	71.1	190.4	88.
41.	5.	71.	21.6.	100.4	38.	130.	54.4	161.	71.6	191.	88.3
42.	5.5	71.6	22.	101.	38.3	131.	55.	161.6	72.	192.	88.8
42.8	6.	72.	22.2	102.	38.8	132.	55.5	162.	72.2	192.2	89.
43.	6.1	73.	22.7	102.2	39.	132.8	56.	163.	72.7	193.	89.4
44.	6.6	73.4	23.	103.	39.4	133.	56.1	163.4	73.	194.	90.
44.6	7.	74.	23.3	104.	40.	134.	56.6	164.	73.3	195.	90.5
45.	7.2	75.	23.8	105.	40.5	134.6	57.	165.	73.8	195.8	91.
46.	7.7	75.2	24.	105.8	41.	135.	57.2	165.2	74.	196.	91.1
46.4	8.	76.	24.4	106.	41.1	136.	57.7	166.	74.4	197.	91.6
47.	8.3	77.	25.	107.	41.6	136.4	58.	167.	75.	197.6	92.
48.	8.8	78.	25.5	107.6	42.	137.	58.3	168.	75.5	198.	92.2
48.2	9.	78.8	26.	108.	42.2	138.	58.8	168.8	76.	199.	92.7
49.	9.4	79.	26.1	109.	42.7	138.2	59.	169.	76.1	199.4	93.
50.	10.	80.	26.6	109.4	43.	139.	59.4	170.	76.6	200.	93.3
51.	10.5	80.6	27.	110.	43.3	140.	60.	170.6	77.	201.	93.8
51.8	11.	81.	27.2	111.	43.8	141.	60.5	171.	77.2	201.2	94.
52.	11.1	82.	27.7	111.2	44.	141.8	61.	172.	77.7	202.	94.4
53.	11.6	82.4	28.	112.	44.4	142.	61.1	172.4	78.	203.	95.
53.6	12.	83.	28.3	113.	45.	143.	61.6	173.	78.3	204.	95.5
54.	12.2	84.	28.8	114.	45.5	143.6	62.	174.	78.8	204.8	96.
55.	12.7	84.2	29.	114.8	46.	144.	62.2	174.2	79.	205.	96.1
55.4	13.	85.	29.4	115.	46.1	145.	62.7	175.	79.4	206.	96.6
56.	13.3	86.	30.	116.	46.6	145.4	63.	176.	80.	206.6	97.
57.	13.8	87.	30.5	116.6	47.	146.	63.3	177.	80.5	207.	97.2
57.2	14.	87.8	31.	117.	47.2	147.	63.8	177.8	81.	208.	97.7
58.	14.4	88.	31.1	118.	47.7	147.2	64.	178.	81.1	208.4	98.
59.	15.	89.	31.6	118.4	48.	148.	64.4	179.	81.6	209.	98.3
60.	15.5	89.6	32.	119.	48.3	149.	65.	179.6	82.	210.	98.8
60.8	16.	90.	32.2	120.	48.8	150.	65.5	180.	82.2	210.2	99.
61.	16.1	91.	32.7	120.2	49.	150.8	66.	181.	82.7	211.	99.4
						151.	66.1	181.4	83.	212.	100.

APPENDIX

THE ARRANGEMENT OF THE LABORATORY AND ITS FURNITURE

THE proper location of the room used for the microscope is a matter strongly insisted upon as important by most microscopists, some contending that a northern exposure is absolutely necessary to insure the proper adjustment of the light, while others assert that a western or southern exposure will do just as well if the windows are covered with white shades so that the light will be uniformly distributed through the room and the glare overcome. The latitude in which the laboratory is situated will have much to do with determining this important question, but there is no doubt of the fact that a northern light produces much more pleasing effects upon the eye of the observer, since the light is distributed from the sky and is softer in its results.

If the laboratory is also to be used for purposes demanding the direct rays of the sun, then a southern exposure will be demanded; in this case the even distribution of the light can be controlled by means of white shades on the windows, adjusted to rollers.

The laboratory should be well lighted with large windows so situated as to supply an ample amount of illumination at all hours of the day. The ventilation must be looked after and put in the best possible condition, so that all deleterious gases from the lungs of the students and from the chemical combinations which may be produced in the laboratory may be carried off as rapidly as possible. It is important to have the heating apparatus so adjusted that the temperature will be normal at all times of the year; in the winter by the regulation of registers, and in the summer by electric fans or other like means. The strictest

attention must be paid to the entrance of dust particles into the laboratory, as nothing so greatly injures apparatus and delicate experiments as this enemy of scientific research. Provision must be made in the laboratory for illuminating and heating gas, and it will be convenient to have gas fixtures on each table in easy reach of the student for many purposes during the preparation of the specimen for mounting. Electric lamps with ground-glass globes mounted on movable bases will be convenient during dark, cloudy days; or, if electricity is not available, Welsbach mantles will give good light with suitable gas-burners.

The room should contain an ample supply of water, with a properly constructed sink located near the centre of the laboratory, or in easy reach of all members of the class. This sink should have several faucets, and it should be lined with lead to withstand the action of chemicals. The laboratory must have shelving for storing the material and apparatus not in immediate use by the students. Individual lockers are necessary, either attached to the tables or grouped together in one portion of the room where the reagents and apparatus of the workers may be safely kept free from dust and interference, and in order that each student may be rigidly held responsible for the outfit. The laboratory locker in use in Cornell University and described in the *Journal of Applied Microscopy*¹ will be found very efficient in this connection. The description given of these lockers is as follows: "The lockers, like all of the woodwork in the laboratory, are of quartered oak. Each is provided with a combination lock, the combination of which is known to the student to whom it is assigned and the laboratory director only. The lockers themselves contain no fixtures whatever, being provided with six pairs of slides. The laboratory owns a large number of reagent boards and drawers, all of which are exactly the same size and of the proper width to fit into the lockers. Each student is provided with as many of these reagent boards or drawers as his work requires, and all reagent bottles, glassware,

¹ *Jour. App. Mic.*, Vol. I, p. 26.

accessory apparatus, material, etc., are always to be kept on these boards or in the drawers. The reagent boards are of light pine one and seven-eighths inches thick, and have on one side depressions in which the bottles or dishes containing reagents, specimens, etc., are set."

The Table. — This necessary part of the furnishing of a laboratory has assumed almost as many shapes and styles as there are laboratories in the country. The form, height, and dimensions are subjects greatly discussed by microscopists; but the general consensus of opinion, however, gives the following rules in regard to the laboratory table:—

1. The student should sit rather than stand at the table.
2. Stools rather than chairs are preferable, because of the reduced space they occupy, and the ease with which the student can move to and from the table. Stools with revolving seats adjustable for height are best.
3. When drawers are used in the tables, combination locks are preferred for many reasons that are obvious to the thoughtful laboratory director who has had experience in the use of keys which are half the time in a "lost" condition.
4. The tops may be either of wood, slate, or glass, to suit the whim of the instructor. The advantages and disadvantages of each will be given farther on.
5. If the space in the laboratory will permit, it is best to give each student a table to himself. In small laboratories this would not be feasible. Care should be taken, however, not to crowd the manipulators.
6. Each student should have a drawer in the table for his own use, or a locker in the near laboratory within convenient reach, in which he may secure the delicate dissecting instruments furnished him by the director.

The experience of the American microscopists has developed a variety of tables, which may be classified under the three following groups:—

1. Simple small tables suitable for two undergraduate students. The tops to be 20 × 54 inches in dimension.

2. The table shown in Figs. 125 and 126 is constructed for two students. It contains drawers at *A*, *B*, *F*, and *G*; compartments at *C*, *D* for the microscopes when not in use; shelves at *E*, *E'*, with doors opening at each end of the table at *H*.

3. Figure 127 shows a table popular in many laboratories in this country. The lower portion of the figure represents a top view of the table, and the upper part a side view. The dimensions are given in the illustration. This table is constructed to

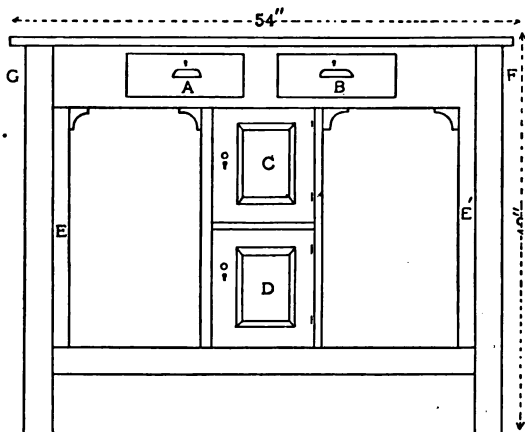


FIG. 125.—Laboratory Table, Side View.

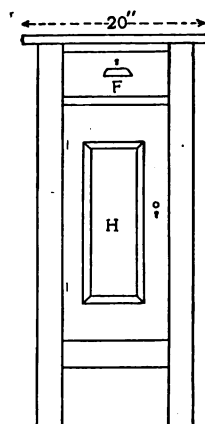


FIG. 126.—End View.

accommodate seven students, and the shape will permit the light from the window to reach each microscope without interference by the neighboring students.

The material used in the construction of the tops of the laboratory tables is also a matter of considerable controversy among microscopists. Wood, slate, and glass have each earnest and strong advocates. If wood is used, the top must be prepared to prevent its disfigurement by stains and other chemicals which will soon mar its surface and render it unsightly and unpleasant to the instructor, whose motto should be, a clean laboratory in all of its approaches and appurtenances. The following methods of procedure are recommended to give impervious surfaces to

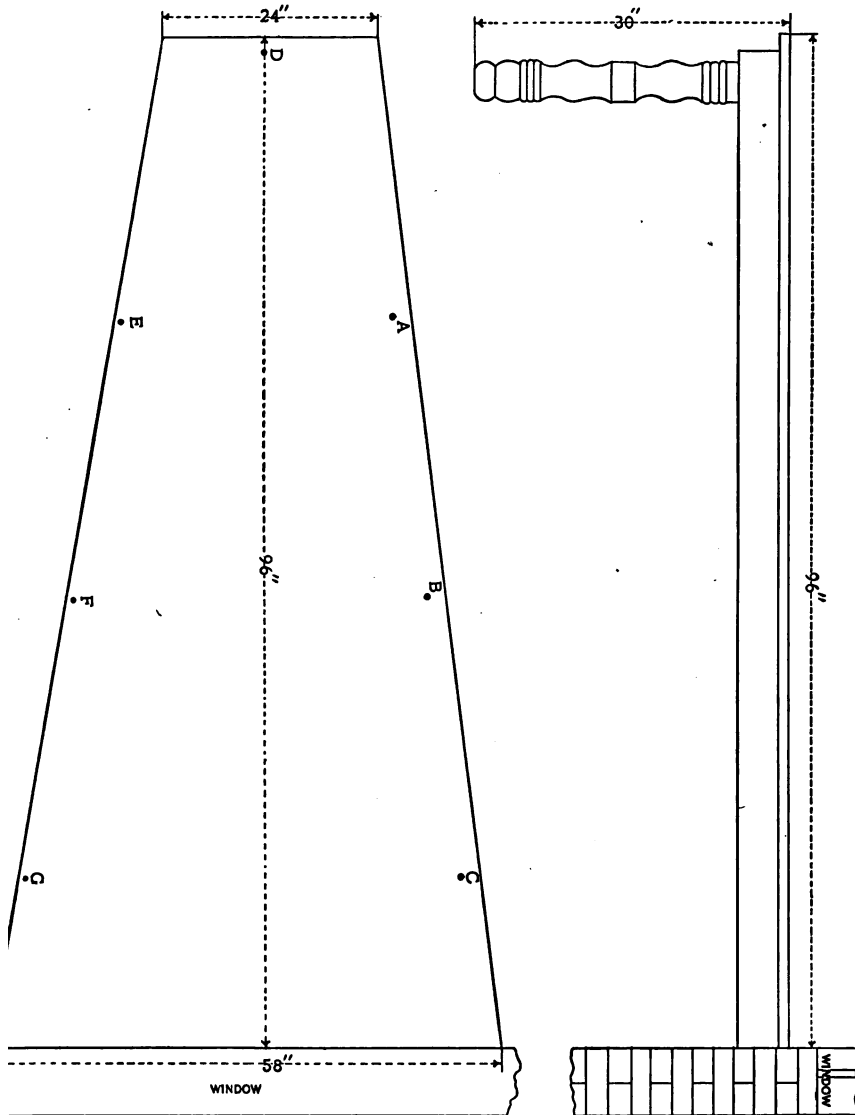


FIG. 127. — Laboratory Table, Top and Side Views.

the action of acids and other chemicals generally used in the laboratory:—

1. This method will produce a black stain and finish which for many purposes is desirable in biological work. To secure this result make two solutions, the following:—

No. 1

Copper sulphate	125 grammes
Potassium chlorate	125 grammes
Water	1000 grammes

Boil until the salts are dissolved.

No. 2

Anilin hydrochlorate	150 grammes
Water	1000 grammes

Or —

Anilin oil	120 grammes
Hydrochloric acid	180 grammes
Water	1000 grammes

Apply two coats of solution No. 1 while hot, and when dry spread on two coats of No. 2, and after the wood is thoroughly dried rub on raw linseed oil. A continued rubbing of the oil will yield a polish, and the deep-black color is produced by washing out the extra chemicals with hot soap-suds. It is claimed for this method that the wood will resist the action of concentrated chemicals.¹

Another method is recommended which will accomplish practically the same results. This has been suggested by Professor Dodge of Rochester University, and consists in making a liberal application to the freshly planed tops of a solution made by boiling logwood chips in an iron kettle. This solution must be concentrated. After the first coat dries the second is applied, which is also followed by a strong solution of sulphate of iron in hot water. When dry the table is thoroughly sand-papered, and hot paraffin of high melting-point (55° to 60° C.) is poured

¹ *Four. App. Mic.*, Vol. I, p. 145.

on. The paraffin is well rubbed into the wood by means of a hot flat-iron. When the surface of the table becomes cool, the superfluous paraffin is scraped off by a thin piece of steel with a straight edge.

Professor F. R. Wright of Cornell University prefers the use of glass tops for tables, and in describing their use in his laboratory he makes the following comments: "The table is covered with corrugated rubber matting, which is of the same size as the top of the table, and on this pad is placed a piece of plate glass which projects about half an inch beyond the edge of the table. The rubber matting prevents the glass slipping, insures to a certain extent against breakage, offers a good background, and takes up much of the vibration. The glass removes the difficulties met with in the use of either wood or slate; it can be thoroughly disinfected, is easily cleaned, and is not unpleasant for one at work."¹

The advocates of the use of slate-top tables claim all the advantages for them that are admitted as belonging to glass, with the additional advantage of cheapness over glass. The microscopists who prefer wood tops claim that glass vessels are easily broken on the slate and glass, and that unless extraordinary care is exercised much damage will result from this source alone in the laboratory. This objection is met, however, with the statement that felt pads may be used on the table upon which to rest the delicate glass, and the neatness in appearance and the cleanliness so easily produced by frequent washing are advantages in favor of slate or glass not to be despised or overlooked.

Photo-micrography.—An adjoining room opening into the main laboratory should be fitted up for experiments in photo-micrography. This room must contain the following furniture:

1. A long, narrow table to hold the camera and microscope and attachments. The centre of the room is the best position for this table.
2. A table to contain the enlarging camera and its outfit.

¹ *Jour. App. Mic.*, Vol. II, p. 231.

3. A stand, similar to a camera stand, to hold the lantern and projection apparatus. This stand should be constructed so that the top can be lowered, elevated, or inclined to suit the character of the picture and the relative position of the lantern and the screen. The "Continental stand" will be found well adjusted for this purpose.

4. A screen sufficiently large to hold the picture projected from the lantern. This screen may be constructed on rollers suspended from the ceiling, to permit of being rolled up as a protection against dust, and to remove it out of the way of other operations.

5. The windows of this room must be provided with opaque shades, mounted on spring rollers, so that the room may be darkened in a few moments when desired. These shades should be made of black material and thick enough to exclude all rays of light. The best way to attach them to the window is to fasten the roller fixtures on the sill and the pulley at the top of the window-frame. The cords, after leaving the pulleys, are run to some convenient point in the room, where they will be out of the way of other appliances and yet so situated as to be conveniently reached. The rollers should be placed in light-tight boxes, and around the window-frame should be fastened a grooved frame, in which the edges of the shade will run when it is raised.

6. For delicate projection direct from the microscope a large ground-glass plate mounted in a frame will be found necessary for satisfactory results.

7. In one corner of this room a dark room may be cut off, in which to perform all photographic work which cannot be done in white light. This dark room should be provided with a sink and a faucet for supplying ample water; shelving to contain such chemicals as are affected by white light; electric lamps covered by ruby globes; and all the apparatus required to develop the sensitive plates.

8. Storage battery to supply the electricity for lighting and heating.

The room should also be provided with the following additional apparatus and conveniences : —

1. Earthenware vessels at each table to contain the refuse and rejected fluids. These vessels should be emptied every day by the janitor and carefully washed clean before returning to the tables.

2. Carrying trays 18" $\times$ 20" for transporting the bottles and glassware needed for the students during the hours of work. There should be a case for these trays near the shelves containing the glassware and bottles of chemicals, so that the assistants may conveniently supply the wants of the students without much trouble.

3. Dissecting pans with wax, cork, or paraffin bottoms will be useful for all work in zoölogy and where injection operations are required.

At suitable places in the laboratory should be located tables for holding permanently the microtomes, paraffin and celloidin apparatus, water-baths, and card indexes of the material belonging to the laboratory.

In the laboratory conducting experiments or studies in subjects relating to zoölogical matters, an aquarium will be found to be a valuable apparatus, to contain the animals, marine and freshwater, in a live condition until needed for the experiment.

Connected with the laboratory should be a ventilating hood for carrying off all noxious and poisonous chemicals used for certain operations.

A greenhouse ought to be an adjunct to the laboratory in which plant studies are conducted, but if this is not possible, then the next best arrangement will be window trays containing boxes for use in growing plants. These trays should be made of zinc to catch the water with which the plants are watered, and prevent its running on the window-sill and on to the floor of the laboratory.

A table for holding blast-lamps, blowpipes, and such like heating apparatus will be a convenience, and sometimes an absolute necessity.

A library attached to the laboratory has become one of the required items in the equipment. If the institution of which the laboratory is a part has a library, even then it is the part of wisdom to transfer to an adjoining room to the laboratory those books which bear directly on the subjects studied, so that the workers may without delay or inconvenience refer to any books for information desired. •

INDEX

- Abbe, Prof., 8, 16, 24.
 Abbe's Substage Illuminator, 15.
 Aberration, Chromatic, 5.
 Absolute Alcohol, 108, 288.
 Acetylene Gas, 171.
 Acid, Acetic, 73, 113, 241.
 Carminic, 107, 118.
 Chromic, 241.
 Chromic Solution, 72.
 Glacial, 73, 107.
 Hydrochloric, 231.
 Lactic, 241.
 Nitric, 228.
 Osmic, 72, 237, 241.
 Salicylic, 287.
 Sulphuric, 84, 180, 190.
 Æquorea, 246.
 Agar-agar, 212.
 Air bubbles from slides, 295.
 Albumen, Mayer's, 70, 100.
 Alcohol, 76, 78, 261.
 Alum, 107, 113, 118, 190.
 Chrome, 180.
 American Thread, 32.
 Ammonia Carmine, 230.
 Ammonium Chloride, 142.
 Amphioxus, 250.
 Amphipods, 248.
 Anaerobic Culture, 220, 281.
 Kasperec's, 221.
 Novy's, 223.
 Wright's, 281.
 Angular Aperture, 34.
 Anilin, Green, 106.
 Anthozoa, 244.
 Aperture Table, 34.
 Aplanatic, 5.
 Apochromatic, 8, 28.
 Apparatus and Accessories, 45.
 Asphalt, 129.
 Aubert, A. B., 107.
 Autoclave, 206.
 Bacteriology, Apparatus used in, 202.
 Study of, 200.
 Bardeen's Freezing Microtome, 93.
 Bausch & Lomb Microtome, 46.
 Bausch & Lomb Optical Co., 25, 29, 49,
 60, 162, 164.
 Beale's Injection Method, 231.
 Beck's Automatic Photographic Lens,
 146.
 Belgian Hone, 52.
 Bell Glasses, 56.
 Benzol, 129.
 Bernhard's Drawing Desk, 136.
 Bismarck Brown, 106, 120.
 Black (Dead) Color, 286.
 Black Polish for Brass, 286.
 Bleaching, 225.
 Blood Serum, 215.
 Blue Stone Hone, 146.
 Bopyrids, 248.
 Borax, 115.
 Born's Method, 237.
 Bouillon, 211.
 Box for Lantern Slides, 195.
 Brachiopoda, 249.
 Brunswick Black Cement, 129.
 Brushes, 56.
 Bryozoa, 249.
 Bugula, 249.
 Cajal, Ramon y, 117.
 Camera, the, 141.
 Cartridge Kodak, 142.
 Long Focus Premo, 142.
 Large Photo-micrographic, Zeiss', 164.
 Camera Lucida, 133.
 Camera Method for making Lantern
 Slides, 191.
 Camera Tripod, 143.
 Campanularia, 246.
 Camphor Water, 75.
 Canada Balsam, 112.

- Canada Balsam, Mounting in, 127, 261.
 Carbutt's Developing Formulæ, 267, 269.
 Carbutt's Dry Plates, 157, 178.
 Carbutt's Fixing Bath, 180.
 Carbutt's Intensifying Bath, 182.
 Care of Eyes, 43.
 Care of Lantern, 198.
 Carmine Stain, 106.
 Celloidin Imbedding, 66, 70.
 Celluloid Films, 145.
 Cements, 127, 261, 295.
 Cerianthus, 245.
 Chaetopods, 248.
 Chloride of Gold, 188.
 Chromatic Aberration, 5.
 Cirripedes, 248.
 Cladocera, 248.
 Clarifying, 80.
 Clark's Lens, 146.
 Clavellina, 249.
 Cleaning Fingers of Stains, 285.
 Cleaning Formulæ, 262.
 Cleaning Glass, 260.
 Cleaning Hands of Cement, 288.
 Cleaning Negatives of Stains, 290.
 Clearing, 80.
 Clearing Agents, 80.
 Anilin Oil, 80.
 Bergamot Oil, 80.
 Cedar Oil, 80.
 Clove Oil, 80.
 Origanum Oil, 80.
 Turpentine Oil, 80.
 Clove Oil, 8, 80.
 Coarse Adjustment, 12.
 Coddington Lens, 2.
 Color Screen, How to make a, 176.
 Colt's Arc Lamp, 168.
 Colt's Lantern, 197.
 Comber, T., 173.
 Comparison of English and French Measures, 300.
 Compound Staining, 110.
 Bismarck Brown — Methyl Green, 110.
 Carmine — Anilin Blue, 110.
 Dahlia — Eosin, 110.
 Eosin — Methyl Green, 110.
 Gentian Violet — Eosin, 110.
 Hæmatoxylin — Eosin, 110.
 Methyl Violet — Eosin, 110.
 Safranin — Gentian, 110.
 Safranin — Hæmatoxylin, 110.
 Compensating Ocular, 22.
 Compound Microscope, 1, 9.
 Condensers, 16.
 Congo Red, 112, 120.
 Contact Method for Making Lantern Slides, 191.
 Continental Ocular, 21.
 Copepods, 248.
 Copper Acetate, 75, 108.
 Copper Chloride, 75, 108.
 Copper Sulphate, 77, 242.
 Corals, 245.
 Corrosive Sublimate, 261.
 Counting Apparatus, 223.
 Cover-glass, 128.
 Cover-glass Gauge, 29.
 Cover-glass, Thickness, 28.
 Cramer's Developer, 264, 268, 270.
 Cramer's Dry Plate, 158.
 Cramer's Fixing Bath, 271.
 Crown Glass, 6.
 Crustacea, 248.
 Ctenophora, 247.
 Cucumaria, 247.
 Culture Methods, 279.
 Cunina, 246.
 Cupric Sulphate, 77, 242.
 Cutting Glass, 296.
 Cutting Paraffin Mass, 66.
 Cutting Sections, 84.
 Cyanin, 120, 142, 177.
 Dahlia, 110, 120.
 Dallmeyer's Photographic Lens, 146.
 Damar Cement, 127, 261.
 Dark Room, 183.
 Dark Room Equipment, 183.
 Darlot's Photographic Lens, 146.
 Decalcifying, 227, 293.
 Decapods, 248.
 Decapod Cephalopods, 249.
 Degrees, Tables of, 304.
 Dehydrating, 78.
 Dehydrating Apparatus, Thomas's, 59.
 Delafield's Hæmatoxylin, 69, 113.
 Developer for Lantern Slides, 191.
 Developing Formulæ, 179, 263.
 Developing Negative, 178.
 Developing Pointers, 184.
 Developing Trays, 150.
 Developing Trays, Sizes Desirable, 150.
 Diamond Ink, 288.

- Diaphragm Shutter, 148.
 Diaphragms, Use of, 44.
 Dilution Method of Culture, 207.
 Diphyes, 247.
 Dissecting Needles, 55.
 Dissecting Scalpel, 55.
 Dissecting Scissors, 55.
 Dissecting Stand, Zeiss', 3.
 Dissolving Key, 198.
 Dissolving Key for Oxy-hydrogen Lamp, Colt's, 198.
 Dorids, 249.
 Double Plate-holders, 148.
 Double Staining. *See* Compound Staining.
 Doublets, Wollaston's, 2.
 Drawing, 132.
 Drawing-desk, Bernhard's, 136.
 Dry Mounts, 131.
 Dry Objective, 31.
 Dry Plates, 173.
 Dry Plates, Orthochromatic, 175.
 Drying-racks for Negatives, 153.
 Eastman's Developer, 264, 268.
 Eastman's Plates, 158.
 Eastman's Roll-holder, 144.
 Echinodermata, 247.
 Edwardsia, 245.
 Ehrlich's Fluid, 77, 107.
 Ehrlich's Hæmatoxylin, 113, 115.
 Ehrlich, P., 121.
 Eikonogen, 179.
 Eikonogen Developer, 269.
 Elasmobranchs, 250.
 Electricity, 16.
 Electric Lamps, 168.
 Elsching, A., 92.
 Embryos of Torpedo, 250.
 English Thread, 32.
 Enlarging Easel, 154.
 Entoniscids, 248.
 Eosin, 107.
 Equipment for Dark Room, 183.
 Esmarch's Roll Culture, 220.
 Etching Fluid, 287.
 Eucope, 246.
 Exposing Limits, 177.
 Exposure Meters, 154.
 Eyepiece Micrometer, 27.
 Fine Adjustment, 14.
 Fishes, 250.
 Fixing Agents, 71.
 Alcohol, 72.
 Chromic Acid Solution, 72.
 Fleming's Mixture, 73.
 Glacial Acetic Acid, 72.
 Hermann's Solution, 73.
 Kleinenberg's Formula, 74.
 Mayer's Formula, 75.
 Mercuric Chloride, 76.
 Merkel's Solution, 74, 77.
 Nitric Acid, 72.
 Perenyi's Solution, 72.
 Platina Chloride, 73.
 Schaffner's Solution, 72.
 Fixing and Clearing Baths, 180, 262, 271.
 Fixing Bath Vessel, 151.
 Fixing or Killing, 71.
 Fixing Sections to Glass Slip, 100, 291.
 Fixing Sections to Slip, Huber's Method, 101.
 Eisen's Method, 102.
 Koninski's Method, 102.
 Weigert's Method, 104.
 Flasks, Erlenmeyer's, 59.
 Koch's, 59.
 Flemming's Mixture, 73.
 Flint Glass, 6.
 Focussing Cloth, 148.
 Forceps, 54.
 Formaldehyde, 237.
 Formulæ, 260, 263.
 Fractional Culture, 218.
 Francais's Extra Rapid Photographic Lens, 146.
 Freezing Methods, 93.
 Fuerst's Developer, 270.
 Furniture of Rooms, 307.
 Gas Lamp, 171.
 Gas Pressure Regulators, 210.
 Gelatin, 215.
 Gelatin Nutrient, 211.
 General Stains, 105.
 Gentian Neutral Red, 120.
 Gentian Violet, 107.
 Gephyreans, 248.
 Gerlach's Formula, 106.
 German Thread, 32.
 Glass, Clearing, 260.
 Glass Benches, 56.
 Glass Slips, 128.

- Glycerin, 113, 182.
 Glycerin Jelly Mounting, 131, 261, 262.
 Goerz Lens, 146.
 Gold Chlorid, 188.
 Golgi's Method for Staining, 115, 116.
 Graduate, 151.
 Gram's Method for Staining Bacteria, 118, 285.
 Grenacher's Borax-carmin, 69, 106.
 Ground-glass Varnish, 290.
 Gundlach's Photographic Lens, 146.

 Hæmatein, 112.
 Hæmatoxylin, 112, 113.
 Halsted, B. D., 288.
 Hammer Dry Plate, 158.
 Hammer's Developer, 265.
 Hanging Drop Culture, 218.
 Hardening, 76.
 Haug's Decalcifying Method, 228.
 Heidenhain's Iron Hæmatoxylin Stain, 114.
 Hermann's Formula, 73.
 Heteropods, 249.
 Hoffman's Violet, 107.
 Holothurians, 247.
 Homogeneous Immersion Objective, 31.
 Huber, G. C., 117.
 Huyghenian Ocular, 21.
 Hydromedusæ, 245.
 Hydroquinon, 179, 190.
 Hydroquinon Developer, 267.

 Iceland Spar, 252.
 Illuminating Apparatus, 15.
 Imbedding, 83.
 Imbedding in Celloidin, 89.
 Imbedding in Paraffin, 83.
 Immersion Objective, 31.
 Incubator, 209.
 Index of Refraction, 296.
 Infiltration, 82.
 Injection, 229, 233.
 Ink for Writing on Glass, 286.
 Inoculation, 215.
 Intensifying Solutions, 182, 272.
 "Intra vitam" Staining, 119.
 Iodine, 119.
 Iris Diaphragm, 18.
 Iron Black Polish, 286.
 Iron Sesquioxide, 231.
 Isopods, 248.

 James, F. L., 261.
 Jelinek, O., Herr, 92.
 Jung, R., 97.

 Kaiserling's Preserving Solution, 291.
 Killing, Fixing, and Hardening, 71.
 Killing for Injection, 236.
 King's Cement, 129.
 Kleinenberg's Formula, 74, 247.
 Knauer, F., Dr., 261.
 Kodak, 142.
 Kodak, Folding, 142.
 Köllicker, von, 117.
 Kopsch, Dr., 118.

 Laboratory, Arrangement of, 305.
 Laboratory Table, 307.
 Lacquering Solution, 293.
 Lamellibranchs, 249.
 Lamp, Carbutt's Multum in Parvo, 149.
 Lamps — Electric, Acetylene, Gas, Oxy-Hydrogen, Oil, 165, 171.
 Lantern and its Requisites, 195.
 Lantern, Care of, 198.
 Lantern, Colt's Criterion, with Electric Lamp and Microscope Attachment, 197.
 Lantern-slide Developer, 191.
 Lantern Slides, How to make them, 191.
 Leitz's Microscope, 11.
 Lens, Photographic, 146.
 Light, Polarization of, 252.
 Light-tight Boxes for Sensitized Plates, 152.
 Lillie, F. R., 62.
 Liriope, 246.
 List of Illustrations, vii.
 Lithium Carbonate, 115.
 Loxosoma, 249.

 Macerating Fluid, Schulze's, 288.
 Maceration, 237.
 Magnification, 138.
 Magnifiers, Coddington, 2.
 Mann's Method for Paraffin Imbedding, 86.
 Marine Organisms, Preservation of, 240.
 Marlmann's Preserving Solution, 292.
 Marsh's Bleaching Method, 226.
 Mat for Lantern-slides, 194.
 Mayer, Paul, 121.
 Mayer's Formula, 75.

- Mayer's Method for Fixing Sections to Slip, 100, 262.
 Camalum, 118.
 Hæmalum, 112.
 Chlorine Bleaching Method, 226.
 Measures, Tables of, 299.
 Mechanical Stage, 14.
 Merkel's Solution, 74-77.
 Mercuric Chloride, 76.
 Mercury Bichloride, 76, 182.
 Metal, 190.
 Methyl Blue, 120.
 Methyl Green, 108.
 Methyl Violet, 120.
 Metric Measures, Table of, 299.
 Micrometer, 27.
 Micrometer Eyepiece, 27.
 Micrometer, Filar, 27.
 Micrometer Screw, 32.
 Micrometer, Ocular, 27.
 Micron, 28.
 Microscope Attachment for Lantern, 197.
 Microscope, Compound, 1, 9.
 Microscope, Continental, 7.
 Microscope, How to use, 41.
 Microscope, Single or Dissecting, 1, 3.
 Microscope, Spencer, 13.
 Leitz, 11.
 Microscope Stand with Mechanical Stage, Frontispiece.
 Microtome, Bausch & Lomb, 46.
 Bardeen's Freezing, 93.
 Freezing, 93.
 Hand, 45.
 How to care for, 52.
 Jung's, 97.
 Reichert's, 50.
 Ribbon Carrier, 49.
 Microtome Knife, 52.
 Microtome, Minot's Precision, 49.
 Microtome Sharpening Knife, 52.
 Miller's Scheme for Completing Mount, 69.
 Miller, W. S., 61.
 Minot's Automatic Microtome, 48.
 Molgula, 249.
 Moll, J. W., 86.
 Mollusca, 249.
 Moore's Staining-dish, 109.
 Monochromatic Filter, 275.
 Mono-refrigent Light, 256.
 Morrison's Photographic Lens, 146.
 Mortars, 59.
 Mounting in Canada Balsam, 123.
 Mounting Media, 261.
 Müller's Solution, 115.
 Naples Staining-jar, 109.
 Needle-holders, 55.
 Negative Drying Rack, 153.
 Negative Titling, 297.
 Negative Washing Box, 151.
 Negatives, Defects in, 184.
 Nematelminths, 247.
 Nemerteans, 248.
 New York Plates, 158.
 Nitrate of Silver, 116.
 Nivellating Apparatus, 220.
 Nose-piece, Revolving, 19.
 Novey, F. G., 211.
 Novey's Thermo-regulator, 210.
 Nuclear Stains, 111.
 Numerical Aperture, 33.
 Nutrient Media, 211.
 Obelia, 246.
 Objectives, 30.
 Achromatic, 36.
 Apochromatic, 36.
 List of, 40.
 Magnification of, 40.
 Dry, 31.
 Homogeneous, 31.
 Immersion, 31.
 Oculars, 21.
 Compensating, 22.
 Continental, 21.
 Huyghenian, 21.
 Micrometer, 27.
 Projection, 25, 26.
 Ramsden's Positive, 22.
 Oil, Anilin, 80, 107, 118.
 Bergamot, 81.
 Cedar, 81.
 Cloves, 81.
 Origanum, 81.
 Turpentine, 81.
 Opisthobranchs, 249.
 Orienting, 98.
 Eycleshelmer's Method, 100.
 Woodworth's Method, 98.
 Orthochromatic Plates, 175.
 Orthochromatizing Plates, 175.
 Ostropods, 248.

- Palm Oil Soap, 53.
 Paper Imbedding Box, 64.
 Paraffin Imbedding, 66, 70.
 Paraffin Imbedding Box, 64.
 Paste, 294.
 Pedicellina, 249.
 Perenyi's Formula, 72.
 Perophora, 249.
 Petit's Solution, 75, 108.
 Petri Dishes, 57, 219.
 Philoroglucin, 228.
 Photographic Lenses, 146.
 Photo-micrographs, 156.
 Photo-micrographic Apparatus, 141, 159, 311.
 Photo-micrographic Projection Apparatus, Zeiss', 164.
 Photo-micrographs, How to make them, 141, 156.
 Physalia, 246.
 Picric Acid, 74.
 Picric Alcohol, 74.
 Pipettes, 58.
 Plate Culture, 219.
 Platinic Chloride, 73.
 Polariscopes Adjustment, 255.
 Polarization of Light, 252.
 Polarizer, Zeiss', 254.
 Polarizers, Mohl's Investigations of, 256.
 Pollen Tube Emission, 288.
 Positives, Making the, 186.
 Potassium Bicarbonate, 77, 116, 179, 242.
 Bromide, 179, 190.
 Ferricyanide, 115, 231.
 Iodine, 119.
 Potato Culture Method, 215, 282.
 Premo Camera, 142.
 Preparation of Tissue for Mounting, 66.
 Preserving Media for Plants, 263.
 Printing Formulæ, 277.
 Printing Frame, 152.
 Projection Ocular, 26.
 Prosobranchs, 249.
 Przesmycki, A. M., 121.
 Pteropods, 249.
 Pyrenogenids, 248.
 Pyrogalllic Acid Developer, 264.
 Stain removed from Hands, 285.
 Queen's Pantagraph Lens, 146.
 Ramsden's Positive Ocular, 22.
 Ranvier's Picro-carmin Stain, 107.
 Ravenel, M. P., 212.
 Ray Filter, 150.
 Reduction of Intense Negatives, 274.
 Reeves' Bath, 62.
 Refraction, Index of, 296.
 Refraction of Light, 252.
 Reichert's Thermo-regulator, 300.
 Retouching Frame, 153.
 the Negative, 297.
 Revolving Nose-piece, 19.
 Rheostat, Colt's Adjustable, 169.
 Ribbon Sections, 49, 84.
 Ripart's Solution, 75, 107.
 Roll-holder, 144.
 Room and its Furniture, 305.
 Rosen's Method for Imbedding, 89.
 Ross' Photographic Lens, 146.
 Safranin Stain, 108.
 Sagitta, 248.
 Salicylate of Soda, 100.
 Salpæ, 250.
 Scalpels, 55.
 Schizopods, 248.
 Schott, Dr., 8.
 Scissors, 55.
 Screen, Color, 176.
 Scyphomedusæ, 246.
 Secondary or Non-nuclear Stains, 111.
 Section-lifter, 56.
 Seed Plate Developer and Fixing Baths, 266, 267, 269.
 Seed's Dry Plate, 158.
 Selective Stains, 105.
 Selenite, 256.
 Serpulidæ, 244.
 Shellac, 129.
 Silvering Glass, 289.
 Silver Nitrate, 116.
 Silver Stains removed from Hands, 285.
 Siphonophores, 246.
 Size of Photographic Plates, 290.
 Sliding Objective Changers, 20.
 Society Screw, 32.
 Soda, Carbonate, 179, 190.
 Sulphite Crystals, 179, 180, 190.
 Hyposulphite, 180, 188.
 Sodium Acetate, 188.
 Special Injection Masses, 232.
 Spherical Aberration, 4.

- Spring Clips, 56.
Stage, Mechanical, 14.
Stage, Microscope, 14.
Stain, How to, 109.
Stains, 105, 282.
 (See also under each separate stain.)
Stanley Dry Plate, 158.
Stanley's Developer, 266.
Starch Injection, 231.
Steinheil's Photographic Lens, 146.
Sterilizer, Hot Air, 203.
 Steam, 205.
Sterilizing, 205.
Sterling's Constant Pressure Injection,
 235.
Stoppers, Loosening, 296.
Strasser's Method, 239.
Suter's Photographic Lens, 146.
Swing-out Condenser, Zeiss', 17.
Synopta, 247.
Syracuse Watch Glasses, 58.
Syringe, Injection, 234.
- Table of Contents, 2.
Tables, Useful, 260.
Table-top Finish, 298, 310.
Thermo-regulators, 210, 230.
Thickness of Cover-glass, 28.
Thyone, 247.
Tray, Hard Rubber Developing, 150.
Trematodes, 247.
- Tube Culture, 216.
Tube Length, 10.
Tunicates, 249.
Turbellaria, 247.
Turn-table, 60.
Turpentine, 80.
- Victoria Blue Stain, 108.
Virodteff's Preserving Solution, 291.
Velella, 246.
Vermes, 247.
- Wash Bottles, 58.
Watch-glasses, 58.
Water-baths, 61.
Weigert's Stain for Nervous System,
 114.
Welsbach's Gas Burner, 164.
Wickersheim's Preserving Solution, 291.
Wire Baskets, 209.
Wollaston's Doublets, 2.
Wright's Culture Method, 281.
- Xylol, 70, 129.
- Zeiss, 8.
Zeiss' Anastigmat Photographic Lens,
 146.
Zeiss' Compound Microscope, Frontis-
 piece.
Zeiss' Dissecting Microscope, 3.



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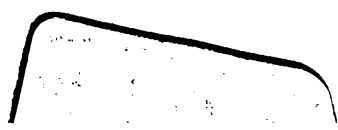
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